A STUDY OF PLASMA ASCORBIC ACID VALUES WITH RELATION TO THE TYPE OF DIET USED IN PUERTO RICO BY GROUPS OF INDIVIDUALS OF WIDELY VARIED ECONOMIC STATUS

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The early work of Ashford ('29) in Puerto Rico and of Rhoads and his associates (Castle et al., '35), as well as of other investigators working elsewhere, has indicated that the sprue syndrome probably has its origin in a dietary imbalance of some sort. In connection with investigations being carried on at the University Hospital with patients exhibiting the sprue syndrome, plans were made to include studies on nutritional status with regard to the vitamins. In order to judge the significance of these findings it was necessary to make companion studies with representative samples of the population not suffering from sprue.

As encountered in Puerto Rico, sprue is found in individuals at all economic levels of living. The majority of cases come, naturally, from the low-income groups and, more especially, from that group having less than enough for satisfactory subsistence since it is the most numerous. The companion studies on non-sprue subjects were therefore planned to include groups of individuals giving sample populations under the two categories: (1) satisfactory income and (2) low income or no income.

During the course of the preliminary work, it became apparent that information on dietary habits was essential to an appraisal of the results to be obtained and a plan was accordingly made to include a record of dietary habits as a part of the study. The investigation as a whole included determinations of plasma carotene, vitamin A and ascorbic acid concentrations and urinary excretion of thiamine, riboflavin and factor F_2 (Holt and Najjar, '42). The present report covers the values obtained for plasma ascorbic acid concentrations of non-sprue subjects and shows how these are correlated with the data on dietary habits.

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METHODS AND PROCEDURES

Determination of plasma ascorbic acid concentration

Plasma ascorbic acid concentration was determined essentially according to the macro method of Farmer and Abt ('34–'35, '36) except for modifications in volume relations for extracting (Kastlin et al., '40).

Five to six milliliters of venous blood were drawn with a clean dry syringe and transferred immediately to a 15 ml. conical centrifuge tube containing 1 drop of a 20% potassium oxalate solution. After centrifugation, 2 ml. of the clear plasma were transferred to a second tube; 2 ml. of water and 4 ml. of 5% metaphosphoric acid solution were added and the tube was centrifuged. Two milliliter portions of the clear centrifugate were then titrated visually against a dilute solution of 2,6 dichloro-phenol-indophenol, care being taken to reach the end point within 5 to 10 seconds. Blank determinations for the metaphosphoric acid solution were always run parallel to the plasma determinations. The dye solution was standardized each day against a standard solution of pure ascorbic acid.

At the University Hospital and its clinics the blood samples were taken with the subjects in the postabsorptive state. Other subjects, who had eaten breakfast before the blood sample was taken, were instructed to omit citrus fruits or other foods containing vitamin C. In most cases the samples were analyzed immediately. When this could not be done they were stored in the refrigerator as the plasma-water-metaphosphoric acid mixture according to the suggestion of Kastlin et al. ('40) and Golden and Garfinkel ('42).

Blood samples from many subjects were taken at clinics some distance from the School of Tropical Medicine. Golden and Garfinkel ('42) reported that plasma in contact with blood cells might be kept at room temperature for as long as 3 hours with only slight loss of vitamin C. Our samples were never kept in excess of this time and the analyses were usually started within considerably less than 3 hours from the time the blood was drawn.

Selection of subjects

Subjects having satisfactory income. The first group under this category was made up of members of the staff of the School of Tropical Medicine and the University Hospital. As most of those selected receive a better than average salary for Puerto Rico, they have been treated together as one group rather than divided on an exact income basis.

The second group was made up of members of the staff and students in nutrition of the Department of Home Economics at the University of Puerto Rico in Rio Piedras. These subjects are of interest as reflecting the effect of nutrition teaching.

A third group, representative of the average citizen having sufficient income to buy food for an adequate diet, consisted of private patients in the University Hospital who came in for checkups or the treatment of minor ailments, and volunteers.

Subjects having a low income or no income. Four groups are included under this division. Two, groups 4 and 5, comprised individuals living in two housing projects of the Puerto Rico Rehabilitation Administration located relatively near San Juan. At the first project, known as St. Just, each householder has sufficient and suitable land for growing food crops; at the second, called Rio Plantation, there is land with each house but it is very poor and not suitable for crops of any kind except a few stunted coconut trees. The condition of the people at this project is most miserable indeed.

A third group, group 6, included individuals selected at one of the public health centers in San Juan.

There is a well-directed outpatient department at the University Hospital and the subjects in group 7 were selected from the patients attending the clinics there. Many of these individuals were sprue cases that had recovered and had received a certain amount of instruction in regard to diet.

Dietary histories

With the members of the staff of the School of Tropical Medicine and the University Hospital, the staff and students of the Department of Home Economics at the University, and volunteers, the following plan was used for obtaining the dietary history: on the day before the blood sample was to be drawn, each individual was given a mimeographed form for recording (1) the foods eaten that day, (2) the foods eaten at breakfast the day of the examination, and (3) the types of fruits, vegetables, salads and other foods included in the diet regularly. In all other cases the personal interview method was used. For the groups at the two housing projects and the one group in San Juan the records were taken by the nurse in charge. From the subjects in the University Hospital and its clinics a complete history was taken covering not only food habits but all aspects of living that might have any significance in the interpretation of results.

RESULTS

Plasma ascorbic acid concentration

A review of the literature indicated that most investigators consider a plasma ascorbic acid concentration in the region of 0.8 mg. per cent as compatible with a state of good nutrition but not necessarily of saturation. Renal threshold values, indicative of saturation, begin at slightly above 1.0 mg. per cent. The range for deficiency is somewhat arbitrarily given as beginning at 0.5 to 0.4 mg. per cent, while it is admitted by some that a person may have a zero plasma ascorbic acid concentration for a long period without clinical signs of scurvy. Clear-cut symptoms indicative of a chronic deficiency of the vitamin are still to be described, although reference is frequently made to sore and bleeding gums in this connection.

The values obtained for the plasma ascorbic acid concentration of the individuals in the seven groups were summarized according to ranges selected on the basis of these considerations and are presented in table 1. The range 0.50–0.79 mg. per cent was used to avoid a too sharp demarcation between the level generally taken as indicative of deficiency and that accepted as implying good nutrition. The use of a single figure for the transition point might have led to an incorrect classification of subjects with values close to this figure. In such cases more than one determination would be required to give the exact classification, a procedure outside the scope of this investigation.

In presenting table 1 attention is called especially to the similarity in the distribution of values for group 3 in the satisfactory income category and group 7 in the low income category. The first group, representing individuals with sufficient money to buy what they needed for good nutrition, gave nearly as many values in the low range as the second group having insufficient income for adequate food or any other essential commodity.

Dietary habits

The detailed data on dietary habits were analyzed on the basis of types of foods listed as common items in the diet. There were certain pronounced differences in the pattern diets for the seven groups that are of interest.

(1) Foods other than fruits and salads

For those in the satisfactory income category rice and beans was a common item in the diet except for the individuals of group 2. Vege-

TABLE 1

Distribution of values for plasma ascorbic acid concentration determined on groups of individuals in Puerto Rico, of widely different economic status.

PLASMA ASCORBIC ACID CONCENTRATION RANGE	INCOME SATISFACTORY				INCOME UNSATISFACTORY							
	Number cases				Number cases							
	Members staff Sch. Trop. Med. and Univ. Hosp. Group 1	Members staff and students Dept. Home Ec. Univ. P.R. Group 2	Private patients Univ. Hosp. and volunteers Group 3	Percent of total	Members of families in P.R.R.A. Housing Projects				Individuals selected by Social Service Worker San Juan Group 6		Patients Outpatient Department Univ. Hosp. Group 7	PERCENT OF TOTAL
					St. Just [1] Group 4		Rio Plantation [2] Group 5					
(milligrams percent)	*(adults)*	*(adults)*	*(adults)*		*(children)*	*(adults)*	*(children)*	*(adults)*	*(children)*	*(adults)*	*(adults)*	
(0.0–0.19)	(1)		(10)	(6.8)	(7)		(16)		(34)		(6)	(30.7)
0.0–0.49	11	6	26	26.7	11	34	18	26	14	36	30	82.4
0.5–0.79	15	14	12	25.4	2	3	5	1			12	11.2
0.8–0.99	16	13	5	47.8							6	6.3
1.0-	17	19	7								7	
Total	59	52	50		50		50		50		55	

[1] Land suitable for food crops.

[2] Land not suitable for food crops.

tables of the starchy variety such as plantain and yautia were used frequently by all. Other types were listed frequently but not by all. Individuals in groups 2 and 3 had plenty of meat, milk, eggs, butter and cheese. These items were listed less frequently by group 1.

For the four groups in the unsatisfactory income category conditions relating to diet were varied. At the St. Just project, group 4, five of the children ate at the nursery and received rice and beans, starchy vegetables, and crackers often; cheese, butter, tomatoes and string beans once in a while. The other children ate at home with their families, and ate a diet which included chiefly rice and beans, starchy vegetables, codfish, and coffee with milk. Meat was eaten not more than once a week. At the Rio Plantation, group 5, all of the children received food at the school lunch room. The menu included evaporated milk, bread, rice and beans, codfish or canned beef once a week, eggs once a week, a starchy vegetable daily and some other vegetable, frequently canned, served as salad. The diet of the adults eating at home was similar to that for group 4 except that they had less food, the coffee was taken black, and meat was almost never eaten. The diet for group 6, individuals selected by a social service worker in San Juan, was similar to that of group 5 while the members of group 7 had a somewhat better diet, more like that of group 4.

(2) Fruits and salads

The data for intake of fruits and salads were definitely related to the plasma ascorbic acid values and were summarized on that basis.

For the range 0.00–0.49 mg. per cent: Individuals of the three satisfactory income groups as a whole ate very little fruit. The children in groups 4 and 5, who ate at the nursery or the school lunch room received fruit or fruit juice once in a while. The other individuals in the four unsatisfactory income groups apparently ate almost no fruit.

For the range 0.50–0.79 mg. per cent: The use of fruit was reported occasionally by the members of groups 1 and 2 and frequently by group 3. Fruit juices were listed by members of group 3 as used frequently but were usually canned pear juice or canned peach juice. The children in groups 4 and 5 received fruit or fruit juice occasionally as mentioned above. The adults in group 4 of this range reported the use of salads. The one adult in group 5 was the janitress at the Public Health unit who included in her diet regularly a vegetable other than a starchy one or tomato soup. Individuals of group 7 reported the occasional use of fruit, chiefly oranges, grapefruits or bananas according to the price.

For the range 0.80–0.99 and above: Practically all individuals having a plasma ascorbic acid concentration greater than 0.80 mg. per cent reported the regular use of citrus fruits while they were in season and the frequent use of salads.

DISCUSSION

Plasma ascorbic acid concentration

In this investigation plasma ascorbic acid values were determined for 366 subjects. Of these, 161 comprised a group with sufficient income to enable them to have an adequate diet. Of the remaining 205, some had sufficient income for no more than a subsistence diet while the majority had little or no income. Of the 161 subjects, 43 or 26.7% had plasma ascorbic acid values within the range 0.00–0.49 mg. per cent and of the 205 remaining subjects, 169 or 82.4% came within this range.

All of the subjects studied were following their usual mode of living and there is no reason to believe that additional determinations, previous to or subsequent to the one made, would have given values differing from it by a significant margin. With the exception of the two groups made up of the members of the staff of the School of Tropical Medicine and University Hospital and the members of the staff and students of the Department of Home Economics at the University, all subjects were either patients under a doctor's care or who had been seen by a public health nurse or doctor at a clinic. As far as is known, none of them had been diagnosed as having symptoms of scurvy. Certainly none of the subjects in the two groups noted above, as exceptions, had any of the symptoms usually associated with scurvy and only one individual complained of bleeding gums.

A check on the question of the presence of subclinical symptoms of scurvy was obtained through the examination, by one of the authors (S), of all members of one group for signs of nutritional deficiency. These individuals, group 5, had been judged to be in the poorest condition nutritionally of all of those studied. Although many of these subjects had signs characteristic of undernutrition, none showed signs of perifollicular hemorrhages. There were no cases of echymosis or purpura and only occasional signs of gingivitis. In other words, there were no symptoms of scurvy or of severe vitamin C deficiency unless we accept as a symptom pyorrhea and loss of teeth from a poor condition of the gums. These were of frequent occurrence. There were several individuals with almost perfect sets of teeth and several with only one or two teeth missing from sets that showed practically no decay thus presenting contradictory evidence.

In the presentation of the results obtained for plasma ascorbic acid concentration a brief discussion is given of the more or less generally accepted concept of the state of vitamin C nutrition in relation to this factor. In their excellent paper on chemical methods for determining vitamin C deficiency Kastlin et al. ('40) go so far as to describe a plasma ascorbic concentration of 0.70–0.4 mg. per cent as indicating a mild deficiency of vitamin C and anything less than 0.40 mg. as associated with a state of severe deficiency.

In view of the evidence obtained with subjects in Puerto Rico there seems to be justification for asking what is meant by a mild deficiency of vitamin C? A severe deficiency of vitamin C? The further question is also raised: Are present methods for determining vitamin C requirement either sound or adequate? It would seem that answers to these questions can be had only through more complete and extensive studies, than have yet been made, of subjects having plasma ascorbic acid values in what is now commonly described as the deficiency or near-deficiency range.

TABLE 2

The effect of administration of ascorbic acid on the plasma ascorbic acid concentration. The administrations began after the initial value of 0.10 mg. % had been obtained on March 2nd.

DATE	PLASMA ASCORBIC ACID CONCENTRATION	ASCORBIC ACID SUPPLEMENT GIVEN DAILY	
		From — To	Amount
	mg. %	*dates*	*mg./day*
March 2	0.10	March 2–10	50
March 11	0.31	March 11–24	50
March 25	0.56	March 25–April 21 (except 9 days)	50
April 22	0.44	April 22–May 5	50
May 6	0.53	May 6–20	50
		May 23–26	50
May 27	0.43	May 28–June 9	100
June 10	0.98	June 10–23	100
June 24	1.37	June 24–July 21	00
July 22	0.27		

Further support for the justification of these questions was given by additional data collected during the course of the present study from one of the subjects, a clerk at the School of Tropical Medicine. On the first examination this man showed a plasma ascorbic acid concentration of 0.10 mg. per cent. Following the examination he was given a daily supplement of ascorbic acid over an extended period and plasma ascorbic acid determinations were made periodically with the results shown in table 2.

While receiving the supplements his plasma ascorbic acid concentration gradually increased to 1.37 mg. per cent but during the month following discontinuance of the supplement the concentration fell to 0.27 mg. per cent. This man, although small and underweight, is vigorous and healthy. His teeth are in good condition and there has been only one extraction and a few minor fillings. The mainstay of his diet is rice and beans and he eats little if any fruit.

Animals for experimental work are maintained in an adequately equipped animal house close by the School of Tropical Medicine. Under the conditions at hand blood samples for vitamin C analysis could be readily obtained from several species of animals and it seemed of interest to obtain these data in the belief that they might have a possible bearing on the question of plasma ascorbic acid concentration as an index of vitamin C deficiency. The values are given in table 3.

TABLE 3

Plasma ascorbic acid values for different mammalian species.

ANIMAL	PLASMA ASCORBIC ACID CONCENTRATION
	mg. %
Dog	0.25
Goat	0.46
Guinea pig	0.56
Horse	0.46
Monkey	0.41
Rabbit	0.41

Without exception all values fall within the deficiency or near deficiency range described for man. All of the animals from which blood samples were taken were receiving what was believed to be an adequate diet and were healthy in all obvious respects. Unless we wish to assume that the plasma ascorbic acid concentration indicative of good nutrition is higher for man than for other species of animals these results seem also to justify raising the question of what, if any, level of plasma ascorbic concentration may be taken as indicative of vitamin C deficiency.

Dietary habits

In Puerto Rico the dietary pattern is fairly uniform for the entire population; any modification is a result of insufficient income. For the well-to-do a breakfast consists of juice, cereal, eggs, bread or crackers, and coffee with milk. The main meal of the day includes soup, salad, meat, vegetables, rice and beans, and possibly a "dulce" or other des-

sert. Rice and beans are served by the well-to-do, even though they may not be eaten, and are simply returned to the kitchen to be consumed by the servants.

Among the people, as a whole, there is a preference for canned fruit juices such as apple, pear, peach, and prune juices, and apricot nectar, none of which are produced in Puerto Rico. Pears and apples are also considered among the choice fruits. This seems a little paradoxical in view of the fact that oranges, grapefruits, and pineapples are among the important crops of the Island. Many other fruits, rich in vitamin C, grow in Puerto Rico and might be grown in abundance. Salad vegetables, such as lettuce, tomatoes, peppers, cucumbers, and watercress are also major crops. In spite of this, canned asparagus, canned beets, canned peas, and canned green string beans are all too often used as the prime ingredients in salads.

When money for food is not adequate, fruit and salads are among the first items omitted from the diet. Breakfast is reduced to bread or crackers and coffee with milk. In the main meal, soup may still persist and may include most of the vegetables in the menu, except the starchy ones. Served in this manner, the vegetables have lost whatever vitamin C they may have had. The starchy vegetables are usually peeled and boiled, which means that they have lost much of their nutritive values. Rice and beans become a dish of major importance; meat is often replaced with salt codfish.

In the last extremity, the breakfast is reduced to crackers and coffee, which is usually black. There is only one other meal in the day, that eaten late in the afternoon. The items from which this is selected are rice and beans or rice alone, a boiled starchy vegetable or plaintain, and salt codfish.

From this brief survey it should be readily apparent that vitamin C is among the first of the nutrients to be reduced in amount in the diet of the Puerto Rican when money for food becomes inadequate.

SUMMARY

Plasma ascorbic acid determinations have been made for a total of 366 subjects, all citizens or long-time residents of Puerto Rico. These subjects were selected on the basis of seven groups of not less than fifty individuals each according to the income categories: (1) satisfactory income and (2) low income or no income. Information on food eaten the day preceding the examination and on dietary habits, in general, was also obtained.

The data from each of the seven groups were summarized on the basis of ranges for plasma ascorbic acid values usually accepted as indicating (1) severe deficiency of vitamin C, (2) near deficiency, (3) satisfactory nutrition, and (4) good nutrition.

Of the 366 subjects examined, 212 or 57.9%, had plasma ascorbic acid concentration values in the severe deficiency range. The high percentage of values in this range from one of the satisfactory income groups is emphasized as indicating that low income is not the only cause of vitamin C undernutrition.

The information on dietary habits reveals a close correlation between plasma ascorbic acid concentration and the presence of fruits and other vitamin C-rich foods in the diet.

The relation of level of plasma ascorbic acid concentration to deficiency of vitamin C and the requirement of this vitamin is discussed.

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DIETARY ANEMIA IN THE PIGEON

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FOUR FIGURES

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Dietary anemia in the pigeon has been studied previously by several investigators. Barlow ('27, '30) observed an anemia in pigeons fed polished rice; he attributed this to the result of inanition, which complicated the vitamin B_1 deficiency. Dameshek and Myerson ('40) used a ration of polished rice supplemented with 50 μg. of thiamine per bird daily. The anemia that developed in these pigeons responded to the administration of yeast concentrate or relatively crude liver extract, but a highly purified liver extract gave only partial cures. Extensive studies of pigeon anemia have been made by Hogan, Richardson, Johnson and Nisbet ('40), and Lee and Hogan ('42), using a purified basal ration deficient in the vitamin B-complex but supplemented with crystalline thiamine. These investigators reported that for complete recovery of both hemoglobin levels and body weight three different crude fractions are required: a fuller's earth adsorbate of rice bran extract, a fuller's earth adsorbate from crude liver extract, and the liver filtrate fraction.

The present study was undertaken with the object of obtaining further information on the nature of the unknown factors required for hemoglobin formation in the pigeon.

METHODS

Pigeons bred for racing were used although some birds of mixed breed were included. They were housed in individual wire cages fitted with one-half inch mesh screen.

In a preliminary experiment, a group of 10 pigeons was fed polished rice, supplemented with salt mixture (U. S. P. no. 1) 0.1 gm. per day, Labco casein 1 gm. per day, cod liver oil 0.1 ml. per day, and cottonseed oil 0.3 ml. per day. The supplements were administered in gelatin capsules. In all subsequent experiments a purified diet similar to that

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of Hogan and co-workers was used. The diet fed had the following percentage composition: casein (Labco) 20, sucrose 73, salt mixture (U.S.P. no. 1) 4, and cottonseed oil 3. U.S.P. cod liver oil was given once weekly by capsule, 0.8 ml. per bird. The basal diet and water were kept before the pigeons at all times; no grit or other material was given.

Blood samples were taken at weekly intervals from one of the small peripheral wing veins. Acid hematin solutions were prepared for estimation of hemoglobin by the method of Schultze and Elvehjem ('34). The colorimetric readings were made in a Klett-Summerson photo-electric colorimeter (filter no. 42) which had been standardized with a series of blood samples of which the hemoglobin contents were obtained by the oxygen capacity method of Lundsgaard and Möller ('22). In the earlier studies weekly erythrocyte counts were also made with Hayem's fluid as diluent.

RESULTS

Normal levels of hemoglobin and erythrocytes in pigeon blood

In the majority of the pigeons the original hemoglobin values ranged from 14 to 18 gm. per 100 ml. The erythrocyte levels for 30 pigeons were from 2.98 to 4.16 million per cu. mm., with an average value of 3.65 million.

Preliminary experiments with a polished rice ration

A group of 10 pigeons, 1 to 9 years in age, were fed polished rice supplemented with casein, salt mixture, cottonseed oil, and cod liver oil as described above. In accordance with the technique of Hogan et al. ('40), none of the vitamins of the B-complex were given for the first 30 days of the experimental period; during this time the weights of the pigeons decreased 26 to 36%. At this point 5 birds were fed 50 μg. of thiamine per day, whereas 5 received a daily supplement of 50 μg. thiamine, 60 μg. riboflavin, and 30 μg. pyridoxine. In 8 of the 10 birds the hemoglobin and erythrocyte levels decreased during the first 40 days. The average fall in hemoglobin was 81%, and in erythrocytes 85%. However, very surprisingly, within 1 or 2 weeks after the administration of thiamine was begun, either alone or in combination with riboflavin and pyridoxine, hemoglobin and red cells began to rise rapidly toward normal levels. Most of the weight lost was also regained, although at a somewhat slower rate. In none of the pigeons was there

any further fall in hemoglobin. Although the experimental period was extended to 167 days, the blood levels remained normal and the body weights were maintained well throughout the period of study.

Since anemia was not maintained under these conditions, probably because of the polished rice in the ration, the diet was modified accordingly.

Production of anemia with a purified diet

Experiment no. 1. The use of the purified diet described above, proved to be successful in inducing anemia. Twenty pigeons were fed this ration; 5 were given 50 μg. of thiamine each per day. The remaining pigeons were given 50 μg. thiamine, 60 μg. riboflavin, and 30 μg. pyridoxine per bird per day. The majority of the surviving birds developed a significant anemia (arbitrarily chosen as 11 gm. or less, hemoglobin per 100 ml. of blood) within 126 to 140 days. The hemoglobin values at the low point of the anemia, before treatment, were from 6.5 to 10 gm. per 100 ml., with an average value of 7.9 gm. per 100 ml.

Experiment no. 2. In an effort to obtain more rapid development of anemia in a group of 19 pigeons (birds nos. 31 to 49), the purified diet described above was supplemented with 50 μg. of thiamine daily after a depletion period of 4 weeks. This technique proved very satisfactory. Of the 16 birds which survived for an extended period 14 developed anemia (10 gm. hemoglobin or less per 100 ml.) within 60 to 123 days (with an average of 90 days); thus 74% of the pigeons placed on the experimental regime became anemic.

When a level of 8 to 10 gm. of hemoglobin per 100 ml. was reached, the bird was treated with various preparations to determine the effect on the hemoglobin concentration. In some cases riboflavin and pyridoxine were administered in addition to thiamine for a period of 2 to 4 weeks before the administration of test preparations. This addition of riboflavin and pyridoxine did not cause an increase in the concentration of hemoglobin.

The development of the anemia and effect of treatment is shown for a representative group of pigeons in figure 1. A dose of rice bran concentrate[2] of 1.0 ml. per day was found to cause a rapid increase of hemoglobin (pigeon 43), while doses as low as 0.25 ml. gave responses in some birds (pigeon 36). A rice bran eluate, prepared by the method

[2] Vitab Rice Bran Concentrate, obtained from the National Oil Products Company, Harrison, N. J.

of Lepkovsky, Jukes and Krause ('36), was tested on a number of birds. One ml. of the eluate represented 2 ml. of original rice bran concentrate. This material was effective in inducing hemoglobin production, as indicated by pigeons 43 and 47, figure 1. However, the amounts required were considerably larger than for the original extract. It was found that the elevation in hemoglobin levels produced

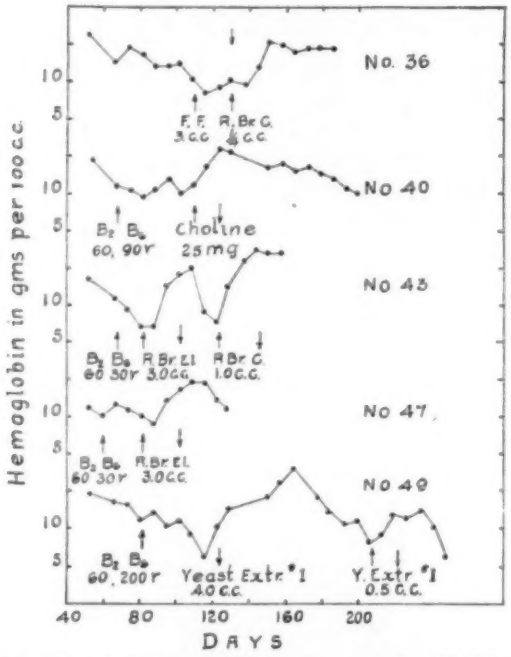

Fig. 1 Hemoglobin values for 5 pigeons in experiment no. 2. All birds received 50 μg. of thiamine per day. Four of the birds received daily supplements of riboflavin and pyridoxine from the time indicated by the symbols B_2 (riboflavin) and B_6 (pyridoxine), the numbers below indicating the daily dose. The therapeutic materials administered were rice bran concentrate (R. Br. C.), rice bran eluate (R. Br. El.), choline, filtrate factor (F. F.) and yeast extract no. 1. The upright arrows (↑) indicate the points at which the administration of the various supplements was begun, while the immediately following inverted arrows (↓) indicate the withdrawal of the supplement. The figures below the symbols indicate the daily dose.

by 2 weeks of treatment was proportional to the dose over a considerable range. The average increases in hemoglobin in 2 weeks produced by daily doses of 0.375, 0.75, 1.5 and 3.0 ml. of the rice bran eluate were 0.0, 1.9, 2.4 and 4.3 gm. of hemoglobin per 100 ml. of blood, respectively. Two pigeons were used for the 1.5 ml. dose, 3 to 5 for the other levels.

Preliminary experiments with a yeast extract were also made on these pigeons. Dried yeast[3] was extracted with 25% alcohol and the extract was concentrated in vacuo. The final volume was adjusted so that 1 ml. represented 1 gm. of yeast (Yeast Extract no. 1). The comparative effects of small and large doses of this preparation are shown in the curve for pigeon 49, figure 1. It is seen that 4.0 ml. given daily for 1 week produced a very large hemoglobin response, an increase of 12 gm. of hemoglobin per 100 ml. of blood being attained in 6 weeks. At a later date a daily dose of 0.5 ml. of the same extract was given to this pigeon for 2 weeks and this elicited a slightly lower hemoglobin increase (3.7 gm.). Further experiments with other pigeons confirmed the activity of this yeast extract in stimulating hematopoiesis (see experiment 3).

To determine the activity of other vitamin B-complex factors a number of the birds, after 100 to 160 days on the experimental ration, were given a daily supplement containing thiamine 50 μg., riboflavin 100 μg., pyridoxine 50 μg., pantothenic acid 200 μg., and nicotinic acid (niacin) 1000 μg. per pigeon. In most birds this dietary supplement produced little or no effect on hemoglobin concentrations and demonstrated that the anemia was not primarily due to a lack of any of these five synthetic factors.

Experiment no. 3. In order to keep the birds in good general condition and to produce a more specific deficiency of the anti-anemia substance, the following plan was used: A group of 36 pigeons was fed the basal vitamin B-complex-free purified diet given above, with no addition of any of the B factors for the first 3 weeks. At this point, supplementation was begun with 50 μg. of thiamine per pigeon per day. After an additional 4 weeks, riboflavin, pyridoxine, calcium pantothenate, and nicotinic acid, in the amounts given in the preceding paragraph, were fed in addition to the thiamine. This supplement was then continued unchanged for the remainder of the experiment.

The 36 pigeons placed on the experiment had an initial hemoglobin level of 15 gm. per 100 ml. of blood, or higher. There was a rapid fall from this level during the first 7 weeks while the birds received none of the B factors other than thiamine. After 7 weeks, the hemoglobin had fallen to levels of 12.0 gm. per 100 ml. or lower with a range of 8.5 to 12.0 gm. in 23 of 34 surviving birds.

In most of the pigeons there was a cure of anemia lasting for several weeks after the administration of the complex supplement containing

[3] Pure dehydrated yeast, Northwestern Yeast Company, Chicago, Ill.

the five synthetic vitamin B factors. Frequently the hemoglobin rose to values as high as or higher than the original levels. After this, the concentration of hemoglobin again declined (figs. 2 and 3). The length of the experimental period required to develop this degree of anemia was 69 to 174 days, most of the birds falling in the range of 83 to 160 days. In this group 4 pigeons were completely refractory to the development of anemia while 7 did not survive.

Effect of therapy with various substances

The anemic pigeons were given supplements when the hemoglobin levels had dropped to 11.0 gm. per 100 ml. or lower. The supplements were usually given for a period of 1 or 2 weeks and were withdrawn as soon as the hemoglobin levels returned to normal. The hemoglobin values thereafter usually decreased again to anemic levels within 1 to 5 weeks after withdrawal of the supplement, so that the birds could again be used as test subjects. The supplements were administered three times weekly in twice the "daily" dose.

The weekly changes in hemoglobin and body weight levels in response to therapy are shown graphically for representative pigeons in figures 2, 3 and 4. The effect of rice bran concentrate, sample 2, is given in figure 2, pigeons 61 and 70. It is seen that a 2.0 ml. dose of this material, given for a period of 1 or 2 weeks, was effective in promoting hemoglobin production in these birds.

The anti-anemic effect of a crude liver extract[4] is shown in figures 2, 3 and 4. It is seen that a daily dose of 100 to 200 mg. of this material is a satisfactory hemopoietic stimulant for most of the pigeons. In the case of pigeon 74, figure 2, a 400 mg. daily dose of the liver powder promoted a satisfactory gain in hemoglobin concentration after a 100 mg. dose had proved ineffective.

Yeast Extract no. 1, described above, proved as successful with this group of pigeons (fig. 3) as in experiment 2. In the case of pigeon 52 it will be observed that a daily dose of 1.0 ml. of this preparation, given for a 1-week period, induced a gain of 4.5 gm. of hemoglobin per 100 ml. in 2 weeks while a daily dose of 0.5 ml. given to pigeon 56 for 2 weeks promoted an increase in the hemoglobin level of 6.0 gm. during a 3-week period.

Inositol, in a dose of 25 mg. per day, was administered to 4 anemic pigeons. It was completely ineffective in all cases (figs. 2, 3; pigeons 74 and 66). Likewise, p-aminobenzoic acid, 15 mg. per day, proved

[4] Wilson's Liver Concentrate Powder 20: 1, from the Wilson Laboratories, Chicago, Ill.

completely ineffective in 5 anemic birds (fig. 4, pigeons 24 and 67). Choline gave a variable response (fig. 2, pigeon 61; fig. 1, pigeon 40).

The response to a liver filtrate preparation is shown in table 1 and figure 3 (pigeon 52). This supplement was made from liver extract powder by solution in water and treatment four times with fuller's earth at pH 4.0 in the proportion of 0.5 gm. of earth per gm. of liver

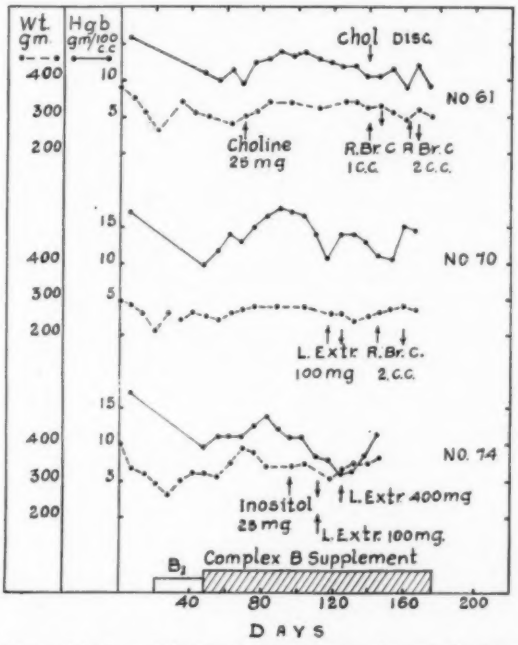

Fig. 2 Hemoglobin and body weight levels for 3 pigeons in experiment no. 3. The vitamin B-complex supplement administered after the seventh week consisted of thiamine, riboflavin, pyridoxine, niacin (nicotinic acid) and calcium pantothenate in the amounts of 50, 100, 50, 200, and 1000 μg., respectively, per pigeon per day. The abbreviated symbols indicate rice bran concentrate (R. Br. C.) and liver extract (L. Extr.). The daily doses appear underneath. The arrows pointed upwards indicate the start of the administration of a supplement. The inverted arrows indicate its discontinuance.

powder for each treatment. While the preparation is definitely less active than the original liver extract, it is seen that much of the activity remains even after repeated treatments with fuller's earth, since a 400 mg. dose of the filtrate produced a satisfactory hemoglobin increase in 2 to 3 weeks in 6 of the 7 pigeons on which it was tested. This indicates that the hemopoietic factor for pigeon anemia, as it occurs in liver extract, is poorly adsorbed by fuller's earth at pH 4.0.

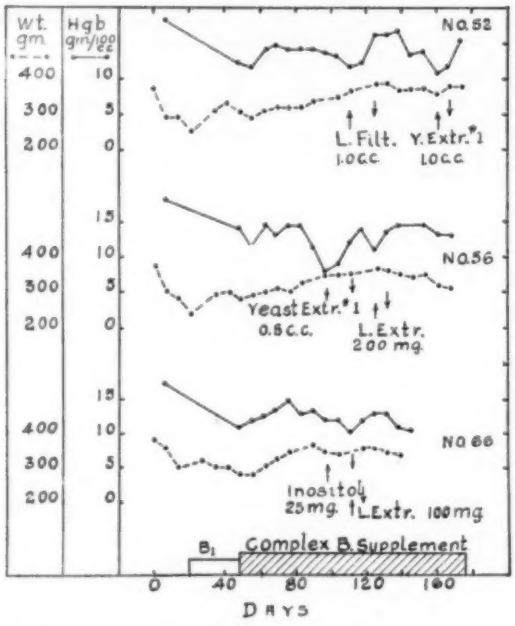

Fig. 3 Hemoglobin and body weight levels in experiment no. 3. The vitamin B-complex supplement was the same as in figure 2. The 1.0 ml. dose of liver filtrate contained 400 mg. of solids.

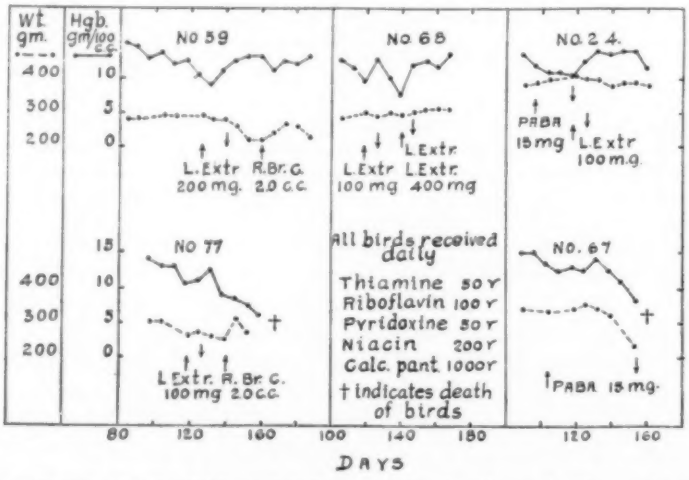

Fig. 4 Response of hemoglobin and body weight levels of anemic pigeons to treatment with liver extract, rice bran concentrate (R. Br. C.) and p-aminobenzoic acid (P A B A).

TABLE 1

Hemoglobin regeneration with liver filtrate preparation.
All pigeons were given a daily dose of 1.0 ml., containing 400 mg. of solids.

PIGEON NO.	DURATION OF TREATMENT	HEMOGLOBIN LEVEL AT START OF TREATMENT	HEMOGLOBIN LEVELS AFTER 1 TO 3 WEEKS TREATMENT			INCREASE IN HEMOGLOBIN LEVELS PRODUCED IN		
			One week	Two weeks	Three weeks	One week	Two weeks	Three weeks
	Weeks	*gm./100 ml.*	*gm./100 ml.*			*gm./100 ml.*		
52	2	11.8	12.3	16.3	16.5	0.5	4.5	4.7
60	3	7.9	11.8	7.2	7.5	3.9	—0.7	—0.4
69	3	10.0	7.1	7.9	15.0	—2.9	—2.1	5.0
72	3	10.3	11.4	11.9	14.8	1.1	1.6	4.5
76	2	12.0	12.7	15.4	13.5	0.7	3.4	1.5
99	3	11.3	10.8	12.6	12.7	—0.5	1.3	1.4
102	2	10.4	14.2	14.0	12.6	3.8	3.6	2.2

Body weight changes

In general, there was little correlation between body weight levels and the state of anemia. Following the period of increased hemoglobin production induced by changing the basal vitamin supplement from thiamine alone to the five synthetic factors, in most birds the hemoglobin levels began to fall fairly rapidly, starting usually some time from the eightieth to the hundredth day. During this period of declining hemoglobin levels the body weight either increased (birds 52 and 56, fig. 3), or remained approximately constant, as in pigeons 61 and 70 (fig. 2) or pigeons 59 and 68 (fig. 4).

The treatment of anemic pigeons with daily doses of 100 to 200 mg. of liver concentrate powder, although effective in raising hemoglobin levels, in most cases had little or no effect on the body weight. This is shown well in the curves for pigeons 24, 59, 68 and 77 (fig. 4). In contrast to this, a 2.0 ml. daily dose of rice bran concentrate in every case produced a 20 to 50 gm. increase in weight during a 2-week period, even though this amount of material supported increased hemoglobin levels in only part of the birds. This is shown in figure 2, pigeons 61 and 70 and in figure 4, birds 59 and 77. These results, along with similar findings on other birds, suggest that there are at least two deficiencies in the basal ration supplemented with thiamine, riboflavin, pyridoxine, nicotinic acid, and pantothenic acid; that is, an anti-anemic factor and a weight-promoting factor.

Symptoms of neuromuscular dysfunction

In a number of birds after a period of 150 days or longer on the deficient ration a state of incoordination developed. The affected

birds were ataxic, i.e., tottered when trying to walk and were unable to fly. Muscular tone was normal, since this condition appeared in birds that were plump as well as those that were emaciated. Occasionally one of the affected birds was observed to have a convulsive attack lasting for about a minute. Following the convulsive episode, the muscles were relaxed. Large doses of thiamine were ineffective in preventing the convulsive seizures. Most of these birds were treated with large doses of yeast extract, rice bran concentrate, or liver extract as soon as the symptoms were observed. Despite this therapy the affected birds invariably died, usually within 2 to 4 days.

DISCUSSION

The prompt response of anemic pigeons fed a vitamin B-complex deficient purified diet to vitamin complex extract from yeast, liver, and rice bran demonstrates the existence of a nutritional anti-anemic factor for the pigeon. Several considerations support this view. One of these is the very rapid increase in hemoglobin levels as the result of yeast or liver extract therapy. An increase of 3 to 5 gm. of hemoglobin per 100 ml. in 1 week is usually obtained with an optimum dose of an active extract. The fall in the hemoglobin level after the withdrawal of the active supplement is frequently almost as rapid as the previous increase. In addition, it may be noted that the factor responsible for the increase in hemoglobin level has very little relation to maintenance of body weight in that frequently pigeons continue to gain in weight while the red blood cell and hemoglobin values are falling. Likewise, doses of liver extract sufficiently large to produce satisfactory hemopoietic stimulation as a rule induce little or no increase in body weight. The data suggest that pigeons require, in addition to the unknown anti-anemia factor, a weight restoration factor.

It has not been possible to identify the anti-anemic agent with any of the known members of the vitamin B-complex. In addition to the vitamins in the basal diet, p-aminobenzoic acid and inositol have been ruled out by therapeutic trial. No experiments were carried out with crystalline biotin or "folic acid."

One must consider the possible similarity of this anemia of pigeons with that described in chickens by Hogan and Parrott ('40) though there may be some differences in behavior of the active principle. In the present study yeast extracts have been very effective in promoting blood formation, whereas 4% of acid-hydrolyzed yeast was used in the anemia-producing diet for the chickens. A further point of difference is that the chick anti-anemic substance is adsorbable on fuller's earth

at pH 1, while it has been found that the pigeon factor in liver extract is very poorly adsorbed on fuller's earth at pH 4.0. It is possible, of course, that this dissimilarity in adsorption may to some extent be due to the difference in pH. Pfiffner and coworkers ('43) have recently reported that the chick anti-anemic factor is identical with Williams' "folic acid."

The possible relation between this anemia of pigeons and that of certain dietary anemias in mammals may also be considered. Anemia which is normocytic, or in a few cases macrocytic, has been produced in several species fed diets lacking the filtrate portion of the vitamin B-complex. Thus Fouts, Helmer and Lepkovsky ('40) found that 6 of 7 dogs lacking filtrate fraction developed a moderate anemia tending toward the macrocytic type. Chick et al. ('38) have reported a normocytic anemia in hogs deprived of filtrate factor. Likewise, Wills, Clutterbuck and Evans ('37) produced a macrocytic anemia in monkeys fed a deficient diet composed of natural foodstuffs and they found that the curative factor in yeast remained in the filtrate upon treatment with fuller's earth.

Thus, the similarity in adsorbability and the common distribution in yeast and liver extracts suggest that the anti-anemic agent in the filtrate fraction of the B-complex active in pigeon anemia may be the same as that reported active in chick and mammalian anemias.

SUMMARY

A severe anemia develops in pigeons maintained on a purified ration containing thiamine, riboflavin, pyridoxine, nicotinic acid, and calcium pantothenate, but lacking other members of the vitamin B-complex. The hemoglobin levels may be restored to normal by administering extracts of yeast, of liver, and of rice bran. The anti-anemic agent in a solution of liver extract remains in the filtrate upon treatment with fuller's earth at pH 4.0.

Evidence is also presented to demonstrate that a second or weight-restoration factor is required by the pigeon.

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COMPLEX CARBOHYDRATES OF SOME CHINESE FOODS

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The relationship of "crude fiber" to the digestibility of foods and intestinal elimination has long been recognized. A report by Crampton and Maynard ('38) shows that for animal feeds the partition of lignin, cellulose and other carbohydrates may have greater biological significance than their total fiber content. Work from this laboratory (Hummel, Shepherd, and Macy, '40), with children, also shows that changes in the proportions of lignin, cellulose and hemicellulose may influence the absorption and retention of nutriments in human beings.

Crude fiber is an important problem in the Chinese diet, which is composed chiefly of foods of vegetable origin and therefore has a high content of indigestible carbohydrates. The published analyses of Chinese foods (Read, Young and Kuang, '37), have shown results obtained with the Weende method which Williams and Olmsted ('35) and Crampton and Maynard ('38) have demonstrated to be inaccurate. Moreover, this method does not provide for partitioning the crude fiber into its different constituents. For these reasons it has seemed worthwhile to reinvestigate the fiber content of Chinese foods.[1]

EXPERIMENTAL

Seventeen commonly used Chinese foods which could be obtained from American markets were analyzed. Three samples of rape and two of mustard were studied to determine the variation in different samples. Some samples were purchased in Detroit early in April, others in New York City early in June. Some of the foods obtained in April cannot be duplicated later in the season. The millet [2] and the rice came from China.

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[1] After completing the complex carbohydrate analyses given in this paper Dr. Kung was recalled to China before she had had an opportunity to finish her manuscript. To extend the value of the paper and add further information on Chinese foods, Harold H. Williams, Ph.D., and D. Maxwell Teague, M.S., determined the proximate composition of the samples available.

[2] The millet sample was supplied by Dr. H. C. Kao of Columbia University, who also supplied, out of season, the sample of celery cabbage dried under similar conditions.

The samples were washed, wiped with clean towels and cut into small pieces which were then mixed. Portions for the cooked and raw samples were weighed immediately, the sample for cooking being weighed directly into the dish in which it was cooked and dried. The Chinese custom of using fat in cooking was omitted to avoid extraction before analysis. The foods were cooked with a small amount of distilled water until they approximated the "doneness" at which they are ordinarily eaten. The green leaves require 5 to 10 minutes, turnip and white gourd about ½ hour. Soybeans were ground, as they are when used in soybean cereal flour mixtures in North China, sufficient 1% bicarbonate solution was added to make a paste, and the mixture then steamed over water. The practice of removing the tough outside layers of the stems of rape and kan lan ts'ai was followed. Cooking water was not discarded and all samples were dried under vacuum at 40°C., after which they were pulverized in a mortar. Since the other vegetables contain little fat, they were analyzed without extraction with ether, which was necessary with soybeans. In the analyses, the method of William and Olmsted ('35) was closely followed.

RESULTS AND DISCUSSION

Except for the dried seeds all of the foods studied had high moisture contents, ranging from 82 to 96%, with the majority above 90 (table 1). For this reason the total fiber of these foods represented only 0.4 to 1.8% of the wet weight, for both raw and cooked samples, although on the dry weight basis the values ranged from 8.5 to 19.2%. Significant amounts of complex carbohydrates were not found in rice, which evidently had been highly polished; the amounts in millet compare with those in the other foods on the wet basis, but are a much smaller fraction of the dry weight; soybeans had a much higher concentration on the wet basis and near the average of the amounts in the other foods on the dry basis.

The three samples of rape showed differences in total complex carbohydrate content which seem attributable principally to variations in the cellulose and water present. The two samples of mustard also showed differences in complex carbohydrate composition and water content. The differences between the fiber contents of samples of the same food are small in comparison with the variations found in other food components, owing to degree of hydration, age, storage conditions, species and soil conditions.

On the wet basis lignin was found in amounts ranging from less than six hundredths (0.06) to 0.5%, however, 1.4 to 3.2% of the dry ma-

TABLE 1
Complex carbohydrate, water, protein, fat and ash contents of Chinese foods. Per cent of dry and wet weights of raw and cooked samples.

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Complex carbohydrate, water, protein, fat and ash contents of Chinese foods. Per cent of dry and wet weights of raw and cooked samples.[1]

FOOD		MOISTURE	LIGNIN		CELLULOSE		HEMICELLULOSE Total		HEMICELLULOSE Stable		TOTAL COMPLEX CARBOHYDRATE		PROTEIN [2]		FAT [3]		ASH	
			Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Leafy vegetables																		
Rape, (Colza) Brassica Chinensis, Linnaeus	Raw	91	2.2	0.2	7.7	0.7	4.6	0.4	2.8	0.2	14.6	1.3	37.3	3.4	3.2	0.3	17.4	1.6
	Cooked	91	2.2	0.2	7.9	0.7	4.1	0.4	2.4	0.2	14.2	1.3	36.9	3.3	..	..	...	..
	Raw	93	2.6	0.2	7.8	0.5	5.3	0.4	2.7	0.2	15.8	1.1	36.7	2.5	4.2	0.3	14.7	1.0
	Cooked	92	2.4	0.2	6.1	0.4	4.6	0.3	2.3	0.2	13.1	1.0	35.6	2.7	2.7	0.2	12.2	0.9
	Raw	93	2.6	0.2	4.8	0.3	5.0	0.4	2.7	0.2	12.4	0.9	34.7	2.5	4.0	0.3	15.2	1.1
Rape stems	Raw	94	1.6	0.1	8.1	0.4	2.6	0.1	2.2	0.1	12.3	0.7	...	..	..	..	...	..
	Raw	94	1.8	0.1	6.9	0.4	4.0	0.2	2.9	0.2	12.7	0.7	25.0	1.4	..	..	...	..
Cabbage, large, Brassica pekinensis, Rupr.	Raw	96	1.4	0.1	9.4	0.4	4.9	0.2	3.4	0.2	15.8	0.7	19.4	0.8	2.1	0.1	13.1	0.6
Kan Lan Ts'ai, Brassica oleracea, Linnaeus	Raw	89	2.6	0.3	6.9	0.7	5.2	0.6	4.0	0.4	14.7	1.6	36.5	3.9	3.3	0.3	17.0	1.8
	Cooked	89	2.4	0.3	5.9	0.7	4.3	0.5	3.1	0.4	12.6	1.4	33.5	3.8	2.9	0.3	14.7	1.7
Kan Lan Ts'ai stems	Raw	92	2.0	0.2	7.4	0.6	4.4	0.3	3.2	0.3	13.7	1.0	26.4	2.0	1.3	0.1	15.5	1.2
Mustard, Brassica juncea, Coss	Raw	89	1.7	0.2	7.4	0.8	3.9	0.4	2.8	0.3	13.0	1.4	36.5	4.0	2.5	0.3	13.9	1.5
	Cooked	88	1.4	0.2	7.8	0.9	3.3	0.4	2.4	0.3	12.5	1.4	36.3	4.2	1.9	0.2	13.6	1.6
	Raw	92	2.6	0.2	6.9	0.6	5.3	0.4	2.6	0.2	14.8	1.2	35.0	2.8	2.4	0.2	18.0	1.5
	Cooked	91	2.4	0.2	5.9	0.5	5.0	0.4	2.6	0.2	13.3	1.1	35.3	3.0	2.0	0.2	17.0	1.5
Leeks, Allium odorum, Linnaeus	Raw	90	2.0	0.2	9.4	0.9	4.0	0.4	3.3	0.3	15.4	1.5	27.6	2.7	..	..	...	..
	Cooked	92	2.2	0.2	9.7	0.8	3.9	0.3	3.4	0.3	15.8	1.3	...	..	..	..	...	..
Coriander, Coriandrum sativum, Linnaeus	Raw	87	3.2	0.4	6.5	0.8	4.3	0.6	2.8	0.4	13.9	1.8	...	..	..	..	...	..
Other vegetables																		
White gourd., Benincasa cerifera, Savi	Raw	96	0.4	0.0	14.2	0.6	4.5	0.2	3.3	0.1	19.2	0.8	17.8 [4]	0.8 [4]	..	..	...	..
	Cooked	96	0.3	0.0	13.6	0.6	3.8	0.2	3.3	0.1	17.6	0.8	...	..	..	..	...	..
Loofah, Luffa cylindrica, Roem	Raw	95	1.0	0.0	4.5	0.2	4.5	0.2	2.3	0.1	10.0	0.4	...	..	..	..	...	..
	Cooked	95	1.0	0.0	5.4	0.2	3.7	0.2	2.4	0.1	10.1	0.5	...	..	..	..	...	..
Turnip, Raphanus sativus, Linnaeus	Raw	93	0.9	0.1	7.9	0.6	3.2	0.2	2.5	0.2	12.0	0.9	13.3	1.0	0.8	0.0	10.0	0.7
	Cooked	93	0.4	0.0	7.9	0.5	2.7	0.2	1.7	0.1	11.0	0.8	13.9	1.0	2.2	0.2	10.2	0.8
Mung bean sprout (root removed)	Raw	94	1.5	0.1	7.8	0.4	5.2	0.3	3.9	0.2	14.5	0.8	41.1	2.3	..	..	...	..
Mung bean sprout (root and bean removed)	Raw	94	1.3	0.1	6.9	0.4	3.4	0.2	2.7	0.2	11.7	0.6	39.1	2.2	1.5	0.1	4.1	0.2
Lotus roots	Raw	82	1.6	0.3	4.8	0.8	2.1	0.4	1.7	0.3	8.5	1.5	10.3	1.8	0.6	0.1	5.0	0.9
	Cooked	82	1.6	0.3	5.1	0.9	1.9	0.3	1.8	0.3	8.6	1.5	9.6	1.7	0.5	0.1	5.0	0.9
Pea pods	Raw	89	1.0	0.1	7.4	0.8	4.8	0.5	3.6	0.4	13.2	1.5	27.5	3.1	1.2	0.1	5.2	0.6
	Cooked	89	1.0	0.1	7.0	0.8	3.8	0.4	2.3	0.2	11.8	1.3	26.1	2.9	1.0	0.1	5.2	0.6
Dried seeds																		
Rice	Raw	8.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.3	6.8	0.4	0.4	0.5	0.5
Millet, non-glutinous	Raw	7.5	0.5	0.5	0.0	0.0	0.9	0.9	0.6	0.5	1.5	1.4	11.5	10.6	4.1	3.8	1.7	1.6
Soybeans, yellow	Raw	4.4	0.4	0.3	4.6	4.4	7.3	6.0	4.8	4.5	12.3	10.7	53.6	51.3	..	..	...	..
	Cooked	4.0	0.4	0.3	4.3	4.1	3.9	3.7	3.6	3.5	8.6	8.2	52.2	50.1	..	..	...	..
Skinned and	cooked	4.0	0.4	0.3	1.9	1.8	3.3	3.2	3.3	3.2	5.6	5.3	54.8	52.6	..	..	...	..

[1] The values have been rounded to one decimal place. Zeros do not indicate an absence of complex carbohydrate, but merely amounts less than one-half of one-tenth per cent.
[2] Nitrogen content times 6.25
[3] Ether extracted.
[4] Pooled sample (raw and cooked).

terial of the leafy vegetables was lignin in comparison with only 0.3 to 1.6% of the other foods. Cellulose composed 0.2 to 0.9% of the wet weight of the foods, excluding the dried seeds, and on the dry weight basis represented from 4.3 to 14.2%. Significant differences in the lignin and cellulose contents of raw and cooked samples were not found.

The total hemicellulose of the vegetables ranged from 0.1 to 0.6% of the wet weight and from 2.1 to 5.3% of the raw dry weight. Cooking reduced the percentage of total hemicellulose in all the foods, by as much as 1% of the dry weight in pea pods. The amounts of labile hemicellulose indicated by the difference between the values for total and stable complex carbohydrate account for the total fiber values being larger than those heretofore published and conform with the results of Williams et al. ('35, '40) which demonstrated that values for available carbohydrate were too high and for unavailable carbohydrate too low.

Of 17 foods common in the diet of the Chinese, all but 3 have extremely high water contents and therefore low energy values. In many areas of China these vegetables comprise almost the entire list of available foods, while in other regions the dried seeds of soy, rice and millet are available. As eaten the vegetables, i.e., fresh vegetables, dried seeds, would, in general, supply complex carbohydrates in amounts comparable to those of the same weight of soy beans. From the data and energy values published by Chatfield and Adams ('40), although obtained with similar American foods, the relative values of the vegetables and the dried seeds in meeting nutriment requirements can be shown. Amounts of the fresh vegetables weighing 12 times an isocaloric amount of dried soy beans would supply not more than one-fourth the fat (possibly including unavailable fatty substances), and one- to three-fourths of the protein but more than the amount of ash. The use of rice to provide calories equivalent to vegetables would decrease the intake of fat, ash and complex carbohydrates, but would greatly reduce the protein intake. Substitution of isocaloric amounts of millet for vegetables would not reduce the fat, ash and complex carbohydrate intakes as much, and would change the protein intake only slightly.

SUMMARY

Values for the lignin, cellulose and hemicellulose contents of 17 foods common in the diets of the Chinese are given on both wet and dry bases, together with their composition with respect to protein, fat and ash.

Although equivalent weights of the vegetable and seed foods, as eaten, would supply comparable amounts of complex carbohydrates, if the energy and protein requirements were met with the vegetables

alone the intake of complex carbohydrates would be eight to ten times larger than a diet which supplied energy and protein with soy beans.

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VARIATION IN THE ASCORBIC ACID REQUIREMENTS FOR SATURATION OF NINE NORMAL YOUNG WOMEN [1]

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Although numerous publications have appeared, a compilation of published data shows that there remains a considerable variation in suggested requirements for the ascorbic acid saturation of normal adults. Much of the variation is due to the fact that several methods of measuring saturation requirements have been used. These methods have included a plasma ascorbic acid method, urinary responses to ascorbic acid in test doses, and "utilization" or "retention" values of the vitamin. Due to the fact that most of these methods are very time consuming, a rather small number of subjects has been studied.

Using the criterion of a urinary response to a test dose of ascorbic acid given after the subject has been maintained on a controlled diet at a definite level of ascorbic acid intake, Belser, Hauck, and Storvick ('39) studied 7 adults, reporting their requirement for body saturation to be from 1.0 to 1.6 mg. per kg. of body weight; Todhunter and Robbins ('40) studied 3 adults by the same method, reporting their requirement for saturation to be 1.6 to 1.7 mg. per kg. per day. The period on a controlled diet followed a saturation period in which the subject was given massive doses of ascorbic acid to insure saturation of the body tissues. In these studies the lowest response to the 400-mg. test dose after known saturation was used as the criterion for saturation for a subject. Since most of these responses were 50% or more of the 400 mg. intake, most later studies use the 50% level as their standard, and it is taken as the criterion for saturation in this paper.

Using urinary responses to the test dose and also ascorbic acid levels in the blood, Storvick and Hauck ('42) reported data on 5 subjects. The amounts of ascorbic acid necessary for tissue saturation (using the 50% level of urinary excretion) for these subjects were 1.33, 1.32, 1.34, 2.85 and 1.51 mg. per kg. of body weight.

[1] From a dissertation presented to the Graduate School of the Virginia Polytechnic Institute by Alice B. Kline in partial fulfillment of the requirements for the M. S. degree.

Saturation tests were reported on 3 subjects by Lewis, Storvick, and Hauck ('43), and again using the 50% level as a criterion, the values given for saturation were 1.35, 1.26 and 1.22 mg. per kg. of the vitamin.

A somewhat different method was used by Purinton and Schuck ('43) to determine requirements for ascorbic acid saturation of 52 women and 11 men. These authors first determined the "fasting" level of ascorbic acid excretion in the urine, after which they gave a 500-mg. test dose and measured the amount excreted in the urine. From the difference they obtained the amount of ascorbic acid retained or "utilized." By this method it was found that 8 women in the age range of 15–20 years required 1.85 mg. per kg., 29 women aged 20–25 needed 1.86 mg., 11 men aged 20–25 required 1.71 mg. and 15 women aged 25–50 required only 1.50 mg. per kg.

Dodds and MacLeod ('44) studied 12 subjects on four different levels of intake within the normal range of requirements. The intakes varied from 32 to 110 mg. and they were not interrupted with massive test doses. It was found that when intakes reached 1 mg. per kg., plasma levels began to increase decidedly. Three subjects were studied for urinary excretion of ascorbic acid on levels of intake of 82 and 107 mg. These subjects were found to be saturated on 82 mg. which if calculated on the per kilogram basis is from 1.23 to 1.60 mg. for the different subjects.

A summary of the data from the above shows that 18 subjects have been studied by the method which used the urinary response to test dose after given levels of intake. Of these, 17 had requirements between 1.0 and 1.7 mg. per kg. of body weight. One subject required 2.85 mg. per kg. for saturation. Other methods of judging saturation requirements have given values of 1.23 to 1.95 for 66 subjects. From these figures it is evident that there is a great deal of variation among individuals in their needs for ascorbic acid for saturation.

EXPERIMENTAL

The object of this study was to measure by a simplified modification of the urinary response to test dose method, the variation among 9 normal young women in the amount of ascorbic acid necessary for body saturation. The subjects chosen ranged in weight from 49.3 to 70.0 kg. and from 60.50 to 68.28 inches in height (table 1). With two exceptions their ages were between 18 and 22 years. The 2 older subjects were 31 and 35 years old.

The general plan of the experiment permitted the women as far as possible to live under their normal conditions. Their meals were served

in the dormitory dining hall but at a special table which was provided for them so their diets could be properly controlled. They were allowed ad libitum consumption of cereals, cooking fats, butter, sugar, coffee, nuts, eggs, cheese and meats (except glandular organs.) A preliminary study of the pasteurized milk served in the dormitory showed that it contained approximately 0.75 mg. of ascorbic acid in the 500 ml. which was the daily allowance of milk in the diet. Since this was such a small amount of the vitamin it was decided to allow ad libitum consumption of prepared dishes containing cooked milk and also ice cream that was purchased from the dairy which provided the milk for the dormitory.

TABLE 1

The age, height, and weight of the nine women used as subjects.

SUBJECT	AGE IN YEARS	HEIGHT IN INCHES	WEIGHT	
			Pounds	Kilograms
I	31	61.50	109.50	49.8
II	21	61.25	103.00	46.8
III	18	63.75	153.75	70.0
IV	35	64.50	121.00	55.0
V	21	62.00	101.50	46.0
VI	21	60.50	142.50	64.8
VII	20	63.00	108.50	49.3
VIII	22	65.00	118.75	54.1
IX	20	68.25	139.75	63.6

It was thought that the small errors involved in the consumption of ice cream and dishes containing cooked milk would be more than compensated for by the willingness of the subjects to remain on the experiment. All dishes served which contained any fruit or vegetables were eliminated from the diet of the subjects. Each subject was served daily throughout the experimental period the following foods which contained small amounts of ascorbic acid: 100 gm. of canned carrots, 100 gm. of canned green beans, 100 gm. of canned apple sauce, 100 gm. of canned peaches and 500 ml. of pasteurized milk. The foods were analyzed at least twice during each experimental period. The indophenol titration method was used as given by Thornton ('38) except that 0.35% oxalic acid was used instead of a mixture of sulfuric and metaphosphoric acids. Preliminary work had shown the former to be as effective as the latter in prevention of oxidation of ascorbic acid. The total amount of ascorbic acid in the experimental diet was fairly constant, varying from 15.43 mg. per day in the first period to 16.15 mg. in the third period.

Urine collections were made only during the 24-hour periods preceding and following the test dose. Since conclusions on saturation are drawn only from the urinary response to test doses, daily analyses of urine were omitted in order to permit the study of more subjects. Analyses were made by the same method used for the foods (Thornton, '38).

Each experimental period consisted of a 5-day saturation period followed by 6 days on the experimental level. During the saturation period 200 mg. of crystalline ascorbic acid [2] was given each subject for 4 days. On the fifth day a 400-mg. test dose was given and if 50% or more of the total intake for that day was excreted in 24 hours, the subject was considered saturated. On the fourth and fifth days of the saturation period the subjects ate the controlled diet containing approximately 16 mg. of ascorbic acid but for the first 3 days of the period the diet was not controlled. If the subject was saturated she was continued on the controlled diet and the experimental level of ascorbic acid was begun. This was continued for 6 days. On the seventh day the 400-mg. test dose was again given and if 50% of the 400 mg. appeared in the urine, it was considered that the level of the previous 6-day intake had been sufficient for the subject's saturation needs. All crystalline supplements were given at breakfast in water which was colored and flavored to resemble orange juice. Since a range of 1.0 to 1.95 mg. per kg. had been found required for most of the individuals studied, a level of 1.8 mg. per kg. was used for the first period in the present study. If the subject was saturated on this level, 1.4 mg. per kg. was given next. If a subject was not saturated at this level it was assumed that her requirement for saturation was between 1.4 and 1.8 mg. per kg. However, if a subject was not saturated at the 1.8 mg. level she was put on an intake of 2.2 mg. per kg. If a subject was saturated at the 1.4 mg. level she was continued on a lower level. Thus, the saturation requirement for any individual could be found by a continuation of the above process.

RESULTS AND DISCUSSION

All 9 subjects were put on the saturation level at the beginning of the experiment but all did not become saturated during this 5-day period. It was interesting to note that out of the 5 subjects who did not excrete as much as 50% of the test dose, 4 were individuals who had not been habitual in coming to breakfast previous to the study. Since the chief source of vitamin C for the day was usually served at breakfast, the

[2] Acknowledgment is made to Hoffman LaRoche, Inc. for a generous supply of crystalline ascorbic acid.

reason for the lack of saturation is obvious. It was, of course, necessary to continue the saturation level for these individuals until the tissues were saturated before experimental levels could be started.

The responses of the 9 subjects to various levels of intake are given in table 2. It may be seen that 7 of the subjects, all except I and VI, were

TABLE 2

Twenty-four hour excretions of a test dose of 400 mg. of ascorbic acid (total intake = 400 + am't. in diet) following two saturation periods and periods of various levels of ascorbic acid intake.

SUBJECT NO.	SATURATION LEVEL			VARIOUS EXPERIMENTAL LEVELS							
	200 mg.			0.6 mg. per kg. body wt.		1.4 mg. per kg. body wt.		1.8 mg. per kg. body wt.		2.2 mg. per kg. body wt.	
	Period	exc'd.	exc'd.	exc'd.	exc'd.	exc'd.	exc'd.	exc'd.	exc'd.	exc'd.	exc'd.
		mg.	%	*mg.*	%	*mg.*	%	*mg.*	%	*mg.*	%
I	1	225	54					89	21	200	48
	2	186	45								
II	1	251	60			165	40	303	73		
	2	357	86								
III	1	240	58			177	43	226	54		
	2	336	81								
IV	1	194	47			187	45	286	69		
	2	352	85								
V	1	294	71			178	43	206	49		
	2	246	59								
VI	1	230	55					165	40	140	34
	2	135	33								
VII	1	254	61			200	48	331	80		
	2	223	54								
VIII	1	336	81	233	56	370	89	218	52		
	2	350	84								
	3	366	88								
IX	1	260	63			191	46	315	76		
	2	237	57								

saturated at the level of intake of 1.8 mg. per kg. When the level of intake was reduced to 1.4 mg. per kg., 6 of these individuals became unsaturated. Thus, for these 6 young women, an intake above 1.4 but not greater than 1.8 mg. per kg. was required for saturation.

Three of the nine subjects had either higher or lower requirements than 1.4 to 1.8 mg. per kg. Subject VIII excreted 89% of the test dose after a level of 1.4 mg. She was continued on a reduced level of 0.6 mg.

per kg. Her response to the test dose after this level was 56% of her intake. Thus, she was still maintaining tissue saturation on this low level. Time did not permit further study of this individual but certainly her requirement was far below that usually recommended. The reasons for such deviation in requirements for ascorbic acid saturation are at present unknown.

Subjects I and VI were not saturated at 1.8 mg. so they were put on a level of 2.2 mg. per kg. On the latter level, subject I excreted 48% of the test dose and subject VI, 34%. It was not possible to continue study of these 2 individuals but it would seem that their requirements for saturation had not yet been met.

Thus, from the present study we find that of the 9 young women, 6 had a requirement of ascorbic acid for saturation of 1.4 to 1.8 mg. per kg., 1 had a saturation requirement of 0.6 mg. or less, and 2 of 2.2 mg. or more. No report in the literature was found of a requirement for saturation as low as 0.6 mg. per kg. and the reasons for such a low requirement for this young woman are not known. Storvick and Hauck ('42) found that 1 of their 6 subjects had a requirement of 2.85 mg. per kg. which is interesting in view of the fact that 2 subjects in the present study were found to require 2.2 or more mg. per kg. for saturation. It is felt that by using the simplified technique proposed in this study, large numbers of individuals could be studied and the extent of variation in requirements for saturation could be learned.

SUMMARY

1. A simplified modification of the method using responses to the test dose for the determination of saturation needs of ascorbic acid was used. Daily urinalysis for ascorbic acid were omitted and only the 24-hour samples of urine before and after the test dose were analyzed. A more liberal intake of ad libitum foods was allowed and the subjects as far as possible lived and ate under their normal dormitory conditions.

2. Of 9 young women, 6 were found to have an ascorbic acid requirement for saturation above 1.4 mg. per kg., but not greater than 1.8 mg. One subject had a requirement of 0.6 mg. or less per kilogram, while 2 had requirements of 2.2 mg. or above per kilogram.

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THE EFFECT OF SPRAY-DRYING AND THE SUBSEQUENT STORAGE OF THE DRIED PRODUCT ON THE VITAMIN A, D, AND RIBOFLAVIN CONTENT OF EGGS

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ONE FIGURE

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The recent and unprecedented use of dried egg in the human diet has made it imperative to evaluate accurately its nutritive properties. The effect of dehydration on the vitamin content of eggs is a major consideration because of the recognized value of eggs as a protective food.

Hauge and Zscheile ('42) found that the spray-drying process caused little or no loss of vitamin A in eggs. Klose, Jones and Fevold ('43) confirmed this finding, and, in addition, observed that thiamine, riboflavin, pantothenic acid, and nicotinic acid were stable during the spray-drying process. They also reported that during subsequent storage some loss of thiamine occurred and vitamin A was lost fairly rapidly, particularly at elevated temperatures.

A study of the effect of spray-drying on the vitamin A, D, and riboflavin content of eggs and the stability of these vitamins during subsequent storage of the dried product was undertaken at the Beltsville Research Center, Beltsville, Maryland, in May, 1942.

MATERIALS AND METHODS

The samples of fresh liquid and dried eggs were obtained through the cooperation of a commercial egg drying plant in Kansas City. In order to insure proper sampling, samples of fresh liquid eggs were taken from the churn and a few minutes later samples of the dried product were obtained. The samples were frozen and shipped under refrigeration to Beltsville.

The vitamin A content of the eggs was estimated by biological assay with rats and by the spectrophotometric method, vitamin D by biological assay with rats, and riboflavin by the microbiological method.

The general procedure for the biological assay with rats was adapted from the method described in the U. S. Pharmacopoeia XII.

For the vitamin A estimations, rats between the ages of 21 and 26 days were collected in groups in screen-bottom cages and were housed in air-conditioned rooms. They were fed only the basal vitamin A deficient diet. After a depletion period of 3 weeks the animals were placed in individual screen-bottom cages. When the rats reached a constant weight, or by the twenty-eighth day, supplementary feeding of the test material was started and continued for a period of 6 weeks. Weekly weights were recorded for all the animals. Because the eggs used were received in three batches over a period of months, it was necessary to make three separate assays. Usually two to six samples of egg material were assayed together. When six samples were to be assayed, ten groups, each containing four male and four female rats were assembled and distributed isogenically according to weight. A sample of egg was fed to each of six of the groups and a reference standard fed at appropriate levels to the other four groups.

Crystalline β-carotene, dissolved in peanut oil and checked for purity and concentration by means of the spectrophotometer, was used as a standard of reference. The standard was fed daily at levels equivalent to 0.5, 1.0, 2.0, and 3.0 International Units of vitamin A. The results obtained from the growth of the groups of rats fed the standard were used to establish a curve of response. The vitamin A content of each egg sample was estimated from this curve.

The "line" test curative method was used for the estimation of vitamin D. The rats were from the same stock colony as those used for the vitamin A assays. The basal rachitogenic diet, no. 2965, of Steenbock and Black ('25), was fed during the depletion period of 21 to 28 days. The vitamin D-depleted rats were then distributed isogenically into groups, and received supplements of U.S.P. reference cod-liver oil and egg material for 6 to 7 days. The animals were killed and tested for the degree of bone healing on the tenth day after supplementary feeding was begun. A curve of response was established from the means of the bone healing values which resulted from feeding different levels of the reference oil. The vitamin D content of the egg samples was estimated from the response curve. (An arbitrary standard scale extending from 0 to 3 with graduations of 0.5 was used in the estimation of the degree of bone healing.)

The procedure used in the spectrophotometric estimation of vitamin A was essentially that described by Madsen and Davis ('38), except that

purified ethyl ether was used instead of petroleum ether to extract the non-saponifiable fraction.

For the microbiological estimation of riboflavin the egg samples were autoclaved in 0.1 N hydrochloric acid at 15 pounds pressure for 30 minutes. After adjustment of the pH to 4.5 with sodium hydroxide the solution was filtered and the filtrate made up to a suitable volume and assayed by the method of Snell and Strong ('39)

EXPERIMENTAL RESULTS

Dehydration did not cause an appreciable loss in the vitamin A content of emulsified whole egg. The results of both biological assays and physical determinations show good agreement between the fresh and dehydrated samples (table 1). However, the values obtained by the biological assay of egg yolk indicate that drying of this material caused some destruction of the vitamin A.

TABLE 1

Effect of spray-drying on the vitamin A, D, and riboflavin content of eggs (all figures on a dry weight basis).

DESCRIPTION OF SAMPLE		VITAMIN A		VITAMIN D	RIBOFLAVIN
		Biological	Physical		
		I. U. per gm.	*μg. per gm.*	*I. U. per gm.*	*μg. per gm.*
No. 1	Emulsified whole egg	61	31	4.1	14
	Spray-dried whole egg	63	31	3.8	13
No. 2	Emulsified egg yolk	95	39	8.1	9
	Spray-dried egg yolk	64	37	8.6	9
No. 3	Emulsified whole egg	50	21	3.3	13
	Spray-dried whole egg	42	22	4.0	14

The experimental results indicate that drying caused little or no loss in the vitamin D and riboflavin content of the eggs. The variations between the values for the emulsified and dehydrated eggs are slight and may be attributed to experimental error.

The decrease in the vitamin A content of stored spray-dried eggs is shown in table 2. The results of both the biological and physical methods show a considerable loss, the results of the former method indicating a much greater loss than those of the latter. This difference is especially noticeable in the case of the samples stored at the higher temperatures.

Vitamin D and riboflavin in the stored spray-dried eggs were quite stable during storage periods of from 4 to 6 months at temperatures up to 98°F. (table 2).

TABLE 2

Effect of storage on the vitamin A, D, and riboflavin content of spray-dried eggs (all figures on a dry weight basis).

CONDITIONS OF STORAGE		VITAMIN A		VITAMIN D	RIBOFLAVIN
Time	Temperature	Biological	Physical		
Months	°F.	*I. U. per gm.*	*I. U. per gm.*[1]	*I. U. per gm.*	*µg. per gm.*
0	..	63	62	3.8	13
4	30	55	58	3.6	12
3	70	33	47	3.8	13
4	98	16	41	3.7	12
6	98	..	33	..	13

[1] Converted from micrograms to International Units by use of the conversion factor 2.

DISCUSSION

As the time and labor involved in the biological assay method for vitamin A preclude its use in routine analysis, the spectrophotometric method was studied with the intention of correlating the results obtained by the two methods. Preliminary studies of emulsified whole egg and spray-dried egg samples showed that each microgram of vitamin A determined spectrophotometrically was equivalent to two International Units estimated by the biological method. The values in table 1 show reasonably good agreement when the factor 2 is used to convert micrograms to International Units.

The same procedure was followed in estimating the vitamin A content of the stored dried egg, but here the agreement between the two methods was not as good as it was in the case of the recently dried egg samples. The values estimated spectrophotometrically compared less favorably with the biological values as the temperature and time of storage were increased. As high results were obtained with the physical method, presumably the substances responsible for the irrelevant absorption increased in the egg samples during the storage period. Therefore the conversion factor established for the emulsified whole egg and the recently dried egg cannot be applied to stored samples.

As the irrelevant absorption is an additive factor, its subtraction from the total absorption of the vitamin A extract should give the absorption due to the vitamin A. Accordingly an attempt was made to estimate the vitamin A by difference. The results are represented by the absorption curves in figure 1.

Curve 1 is typical of the extract of a dried egg sample. The vitamin A in this extract was destroyed by exposure to sunlight, and the residual absorption measured (curve 2). The difference between these two

curves should be the absorption caused only by the vitamin A. The absorption curve of a vitamin A concentrate was determined (curve 5). The same quantity of the concentrate that was used for this curve was then added to the vitamin free extract; absorption measurements gave curve 3. The vitamin A was destroyed in the extract and also in the solution of vitamin A concentrate. Subsequent absorption measurements resulted in curve 4 for the extract and curve 6 for the concentrate. A comparison of curves 5 and 6 shows that the vitamin was com-

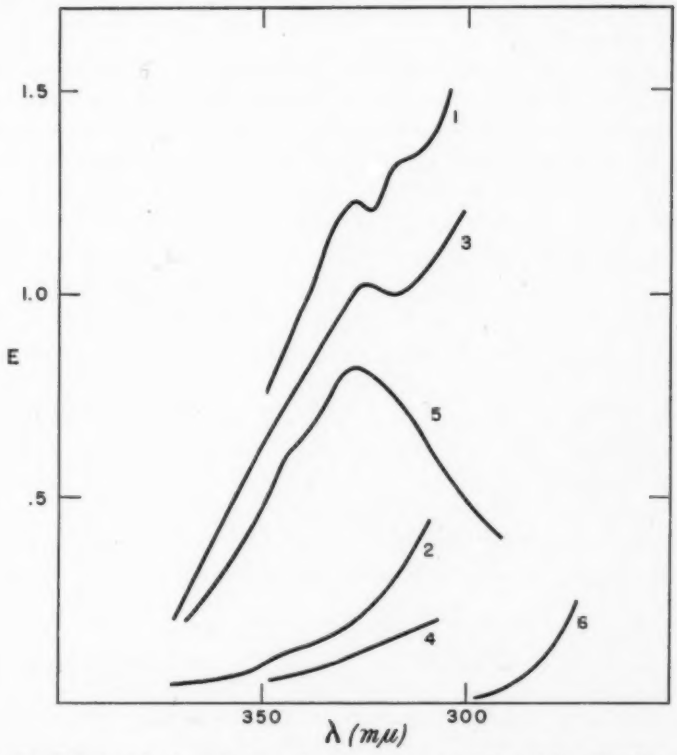

Fig. 1 The effect of irrelevant absorption on the spectrophotometric estimation of vitamin A in stored dried eggs.

Curve 1 Typical absorption curve of extract of stored dried egg.
Curve 2 Absorption curve of extract after vitamin A was destroyed by exposure to sunlight.
Curve 3 Absorption curve of extract after a vitamin A concentrate was added.
Curve 4 Absorption curve of extract after the added vitamin A was destroyed by exposure to sunlight.
Curve 5 Absorption curve of a solution of vitamin A concentrate.
Curve 6 Absorption curve of the solution after the vitamin A was destroyed by exposure to sunlight.

pletely destroyed by exposure to sunlight. As curves 2 and 4 do not show the same intensity of absorption in the region of the vitamin A maximum it is apparent that the irrelevant absorption did not remain constant when the vitamin A was destroyed. Therefore it appears that a conversion factor for each condition of time and temperature of storage would have to be established for the routine analysis of dried egg by the spectrophotometric method.

The retention of vitamin D and riboflavin presents no problem in the keeping of dried egg for the experimental results show that these vitamins are quite stable.

In general the results obtained in this study of the stability of vitamins A, D, and riboflavin in eggs during the spray-drying process and subsequent storage of the dried product are in excellent agreement with the findings of Hauge and Zscheile ('42) and Klose, Jones, and Fevold ('43).

SUMMARY

The vitamin A content of emulsified whole eggs and spray-dried eggs was estimated by biological and spectrophotometric methods, the vitamin D content by a biological method and the riboflavin by a microbiological method. The results indicate that no loss of these vitamins occurred during the spray-drying process. Vitamin D and riboflavin are stable in the stored dried products whereas vitamin A is lost fairly rapidly especially at the higher storage temperatures. Estimation of vitamin A in the stored products by the spectrophotometric method presented difficulties. The effect of impurities that absorb in the region of the vitamin A maximum does not remain constant during the storage of the dried product. Attempts to overcome this difficulty are discussed.

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VITAMIN C CONTENT OF WILD GREENS[1]

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INTRODUCTION

In a recent publication (Stratton, '43), one of the authors has called attention to a number of native wild plants that are useful additions to war-time diets as greens or salad plants. The present paper reports assays of the vitamin C (ascorbic acid) content of a number of the commonest of these edible wild plants, the tests involving both fresh samples, those which had been placed in frozen storage for several weeks before testing, and those which had been cooked.

The literature records tests of vitamin C content for a few species of wild greens in the fresh state. Burrell and Ebright ('40) report assays for this vitamin in dandelion, shepherd's purse, and pokeweed. Burrell and Miller ('39) have also determined vitamin C content in curly dock, wild lettuce, and chickweed. The values for curly dock and chickweed obtained by these workers were comparable to those obtained in this study, but they reported higher vitamin C values for dandelion, shepherd's purse, and pokeweed than were obtained in our determinations. All of these tests were made by means of the dye titration method.

It is well known that the food value of various crops is markedly affected by the environment and soil in which the crop has grown; for this reason an attempt was made to gather the samples for testing in locations characterized by differences in soil, shading, and other ecological variables.

MATERIALS AND METHODS

The species of wild plants tested are listed in table 1. An attempt was made to gather and prepare the plant tissues in ways comparable to the recommended practices in gathering and preparing wild greens

[1] The work reported here was carried out under a joint project of the Oklahoma Agricultural Experiment Station, and the School of Arts and Sciences of the Oklahoma Agricultural and Mechanical College.

TABLE 1

Vitamin C content of fresh, cooked, and frozen wild greens.

SPECIMEN	HABITAT					MG./GM. VITAMIN C, CALCULATED ON:				
						Wet basis			Dry basis	
Plant	Sample	Shade [1]	Soil [2]	Plowed [3]	Succulence [4]	Fresh	Cooked	Frozen	Fresh	Cooked
Curly dock (Rumex crispus L.)	I	±	L	—	S	1.30	1.30	...	13.83	13.83
	II	±	L	+	MS	0.96	0.90	0.23	8.43	7.90
	III	—	BL	—	MS	1.42	1.43	0.33	9.57	9.67
	IV	—	L	—	S	1.55	1.34	0.49	10.76	9.29
	V	—	SC	—	VS	1.26	1.35	0.53	8.64	9.26
	Mean					1.30	1.26	0.39	10.24	9.99
Prickly lettuce (Lactuca scariola L.)	I	+	L	—	VS	0.43	0.37	...	6.32	5.42
	II	±	L	—	S	0.44	0.40	...	3.71	3.35
	III	—	L	—	MS	0.60	0.32	0.04	4.58	2.67
	IV	±	L	+	VS	0.31	0.40	0.23	2.64	3.42
	V	—	SC	—	VS	0.31	0.41	0.11	2.83	3.82
	Mean					0.41	0.38	0.12	4.01	3.73
Poverty weed (Monolepis Nuttalliana (R. & S. Wats.)	I	—	L	+	S	1.18	0.67	...	10.69	6.11
	II	+	CL	—	VS	0.66	0.67	0.13	6.32	6.47
	III	—	L	+	S	0.67	0.67	0.19	4.79	4.79
	IV	—	SC	—	MS	0.81	0.67	0.25	6.11	5.08
	V	—	CL	—	MS	0.67	0.73	0.15	7.50	8.15
	Mean					0.80	0.68	0.18	7.08	6.12
Common chickweed (Stellaria media (L.) Cyrill.)	I	+	L	—	VS	0.24	0.19	0.20	3.03	2.38
	II	±	CL	—	MS	0.33	0.29	0.02	3.30	2.93
	III	—	SC	—	MS	0.35	0.29	0.05	2.95	2.44
	IV	+	L	—	MS	0.06	0.11	0.15	0.61	1.04
	V	—	SL	+	MS	0.34	0.40	0.17	4.20	3.58
	Mean					0.26	0.26	0.12	2.82	2.47
Common dandelion (Taraxacum officinale Weber)	I	±	L	—	S	0.19	0.15	...	1.24	1.01
	II	±	SL	—	S	...	0.19	...	...	1.21
	III	—	CL	—	S	0.12	0.32	0.01	0.84	1.10
	IV	+	L	—	VS	...	0.19	0.01	...	1.75
	V	+	CL	—	S	...	0.21	...	...	1.52
	Mean					0.15	0.21	0.01	1.04	1.32
Red-seeded dandelion (Taraxacum erythrospermum Andrz.)	I	±	L	—	S	0.11	0.14	...	0.76	0.99
	II	—	CL	—	S	0.05	0.53	0.07	0.31	0.16
	III	—	CL	—	MS	0.19	0.22	0.23	1.35	1.56
	IV	+	CL	—	S	...	0.19	...	...	1.39
	Mean					0.11	0.27	0.15	0.81	1.02
Tall dock (Rumex altissimus Wood)	I	—	BL	—	MS	1.58	1.52	0.17	10.56	10.50
	II	—	L	—	VS	1.43	1.59	0.72	13.25	14.67
	III	—	BL	—	MS	1.36	1.17	0.42	10.59	9.16
	IV	—	SL	—	S	1.20	1.31	0.58	9.71	10.57
	V	—	SL	—	VS	1.14	1.37	0.30	8.15	9.78
	Mean					1.34	1.39	0.44	10.45	10.93
Shepherd's purse (Capsella Bursa-pastoris (L.) Medic.)	I	—	L	—	MS	0.68	0.64	0.06	6.04	5.00
	II	+	CL	—	MS	0.65	0.93	0.19	4.96	7.04
	III	—	CL	—	MS	0.73	0.91	...	5.05	6.33
	IV	+	CL	—	FS	0.62	0.61	...	4.92	4.69
	Mean					0.67	0.77	0.12	5.24	5.76
Pokeweed (Phytolacca americana L.)	I	—	SL	—	S	1.28	1.19	0.28	13.38	12.43
	II	—	SL	—	S	1.18	1.16	0.85	11.77	11.62
	III	±	SL	—	S	1.44	1.75	0.35	13.50	16.55
	IV	—	SCL	—	S	1.68	1.70	0.45	15.25	15.40
	Mean					1.39	1.45	0.48	13.47	14.00
Lamb's quarter (Chenopodium album L. I, III, V. Chenopodium leptophyllum Nutt. II, IV.)	I	—	SL	+	VS	0.69	0.99	0.27	5.92	4.74
	II	—	SL	—	S	0.51	0.55	...	5.61	6.12
	III	±	SL	—	VS	0.53	0.41	0.16	4.45	3.43
	IV	—	SL	—	S	0.66	0.88	0.34	4.89	6.30
	V	—	SCL	+	S	0.91	0.94	0.41	7.35	7.62
	Mean					0.66	0.75	0.30	5.64	5.64

[1] Shade: +, full shade; ±, partial shade; —, full sun.

[2] Soil: L, loam; B, black; S, sandy; C, clay.

[3] Plowed: +, plowed within past 6 months; —, unplowed during this time.

[4] Succulence: S, succulent; V, very; M, moderately; F, fairly.

for the table. The majority of the samples were collected between 8 and 11 A.M. and prepared for testing immediately thereafter, before the plants had wilted. An aliquot of each sample was blanched for 1 minute in boiling water, quick-frozen without delay, and placed in frozen-locker storage for a period ranging from 36 to 65 days. A second aliquot was macerated i na Waring Blender and tested without further treatment, while a third aliquot of 50 gm. of greens was cooked for 5 minutes in 100 ml. of boiling, distilled water, after which the assay was made on the combined greens and cooking water. The frozen samples were then treated as the fresh, uncooked samples. The Klett-Summerson colorimeter was used for the ascorbic acid determinations which were made according to the method of Loeffler and Ponting ('42).

DISCUSSION

The vitamin C determinations may be compared with those reported for spinach, in which the vitamin C content is in the approximate range of 0.50 to 0.65 mg. per gm. of greens (Booher, Hartzler, and Hewston, '42). In comparison with this range the wild greens tested fall into three groups: (a) pokeweed, tall dock, and curly dock, with vitamin C contents about double that of spinach; (b) poverty weed, shepherd's purse, and lamb's quarter, which were, roughly, equal to spinach in vitamin C content; and (c) chickweed, common dandelion, red-seeded dandelion, and prickly lettuce with vitamin C values materially lower than spinach.

In general, the changes in vitamin C content following freezing and cooking were comparable to the changes that occur in cultivated greens. With the exception of the red-seeded dandelion the frozen samples showed marked loss in vitamin C content, as is true for spinach. In some cases the vitamin C values obtained for cooked greens were higher than those for comparable samples of uncooked greens. This was particularly true of the two species of dandelion. In some instances no reading was obtained with the raw material, while the cooked material indicated the presence of vitamin C. This may be due to the use of 1% metaphosphoric acid for extraction of the vitamin. Some of these greens may have had higher oxidative activity and Reid ('42) has concluded that higher concentrations of metaphosphoric acid than were used in these tests are necessary for such refractory materials. If this is the case, the vitamin C content of some of our samples may have been materially higher than was brought out by the assays.

The frozen samples varied in their time of frozen storage from 36 to 65 days. Comparison of the loss in vitamin content with length of time

in frozen storage showed no correlation. There was no evidence of a progressive loss in vitamin C content with increasing time of storage within these limits.

The data in table 1 give some indication that plants growing in full sunlight have more vitamin C than those growing in the shade, those in unplowed ground more than those in cultivated land, and those in rich loam or sandy loam soils more than those growing in clay or sandy clay soils. Many factors appear to influence the vitamin C content of wild greens, including soil, moisture, temperature, time of day sample is taken, stage of development of the plant, and others. It was not within the scope of this study to attempt to determine the individual effects of these factors. Such a study would require the growing of plants under controlled conditions.

SUMMARY

Vitamin C assay of fresh, cooked, and frozen samples of edible wild greens showed that pokeweed, tall dock, and curly dock contained twice as much of this vitamin as spinach; poverty weed, shepherd's purse, and lamb's quarter about as much as spinach; chickweed, dandelions, and prickly lettuce considerably less than spinach. Loss of vitamin C in wild greens due to freezing or cooking was comparable to the loss sustained under similar conditions by cultivated greens. There was some indication that the vitamin C content was greater in plants exposed to full sunlight and in fertile, uncultivated soil.

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A STUDY OF CANINE HYSTERIA PRODUCED BY FEEDING CERTAIN BAKED DOG FOODS AND WHEAT GLUTEN FLOUR [1]

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FOUR FIGURES

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The existing knowledge and theories concerning canine hysteria were carefully discussed by Hewetson ('36). He was unable at that time to arrive at any definite conclusions regarding the disease from consideration of the known facts. At best it is an ill-defined malady whose nomenclature, cause, and treatment are in dispute. Numerous other terms have been suggested and used for the syndrome, namely, running fits (Arnold and Elvehjem, '39), fright disease (Grace, '30), epizootic hysteria, enzootic hysteria, infectious hysteria (Campbell, '27), and hyperkinesia (Lintz and Lintz, '29).

The symptoms generally associated with the disease are paroxysmal attacks of hyperexcitability characterized by running, barking or howling, faulty vision, deranged balance, dementia, and often clonic convulsions. The secretion of a heavy mucous saliva is common especially during the convulsions. During the convulsions urination and defecation frequently occur. The affected dogs seem indifferent to injury or to efforts to quiet them. Excitement and physical strain influence the onset and frequency of attacks. Heredity (Berryman and Schlotthauer, '41) is also believed to be a factor affecting the animals' susceptibility. Between attacks the animal may appear normal but is often nervous and apprehensive. Attacks are usually precipitated by stimuli such as a sudden noise or a flash of light.

A number of afflictions have been reported to produce similar symptoms. Melnick and Cowgill ('37) observed hysteria among experimental dogs receiving rations containing gliadin. Arnold and Elvehjem ('39) found that a baked dog food made of wheat flour and meat scrap caused fits in young dogs and thought that a lysine deficiency was involved.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by a grant from Wilson and Company.

They also suggested that a toxicity might be a contributing factor. Patton ('39) attributed similar symptoms in dogs fed a baked dog food to a thiamine deficiency. McGhee ('36) thought that the condition was due to a low blood magnesium level. Chambers ('30) reported a case in which the addition of quantities of tallow to a ration of meat and baked bread cured hysteria. Others have believed that distemper (Grace, '30), ear mites (Chambers, '30), intestinal obstruction (Browning, '29), vitamin A deficiency (Walton, '33), or ticks (Macfie, '30) were the cause of the disease.

Many of the workers who believe the disease to be related to the nutrition of the affected animals have commented on its frequent association with the feeding of baked dog foods. For this investigation it was originally intended to select commercial baked dog foods on the basis of their hysteria-producing potency and secure sufficient quantities of the worst offenders for further experimentation. In the midst of this program, Dr. H. B. Parry,[2, 3] who had been conducting feeding experiments with gliadin in this laboratory discovered that by feeding commercially produced wheat gluten to young dogs all of the usual symptoms of canine hysteria could be produced with great rapidity, and intensity. The frequent occurrence of wheat products as an ingredient of baked dog foods, and the similarity of the symptoms suggested that the disease was the same in both cases. The use of wheat gluten offered a more productive approach to the problem than the feeding of commercial dog foods and subsequent efforts were directed to the investigation of this material.

EXPERIMENTAL

Animals

Mongrel dogs were obtained at weaning and fed a milk diet for several days. During this interval they received as a vermifuge ¾-grain of santonin and ¾-grain of phenolphthalein following a 12-hour starvation period, after which they were transferred to a normal, adequate, dry ration for 1 week before being used for experimental purposes. The animals were kept in a heated, lighted room in individual cages of heavy wire mesh, the floors of which were of concrete covered with wood shavings. The proximity of the animals to one another was such as to permit ready transmission of the malady if an infectious agent were responsible.

[2] Unpublished data.

[3] Present address: Imperial Chemical Pharmaceuticals, Entebbe Uganda, British East Africa.

At the end of each experiment animals which had received hysteria-producing rations were transferred to a normal ration for a recovery period of at least 1 week. By this procedure it was possible to use each animal for several experiments. After animals had attained the age of 6 to 8 months their susceptibility was so reduced as to make them unsatisfactory for assay purposes.

Production of canine hysteria with commercial baked dog foods

The baked dog foods used in this portion of the work were regular commercial products. They were coarsely ground and 97 parts of the ground material mixed with 1 part cod-liver oil, 1 part NaCl, and a part Liver Fraction B [4]. Preliminary experiments had demonstrated that the above supplements did not prevent the occurrence of attacks of canine hysteria. The above rations and water were supplied ad libitum.

TABLE 1

Canine hysteria from the feeding of baked dog foods.

SAMPLE	RESULTS	SAMPLE	RESULTS	SAMPLE	RESULTS
1	fed 18 days; no attacks	6	first attack on 14th day	10	first attack on 24th day
2	fed 34 days; no attacks	6	first attack on 5th day	10	first attack on 11th day
3	fed 41 days; no attacks	7	fed 83 days; no attacks	11	first attack on 32nd day
5	fed 34 days; no attacks	9	first attack on 5th day	11 plus 5% casein	first attack on 28th day
		9	first attack on 5th day		

Results obtained on nine samples are listed in table 1. Limited quantities of sample 1 prevented a more prolonged assay. Two samples were found to cause attacks in less than a week. Two other samples also caused hysteria but more slowly. Five samples failed to show activity. The affected animals displayed symptoms of nervousness, fright, running, howling, and in some cases convulsions. Secretion of a heavy mucous saliva occurred during attacks. The loss of sight which occurred was not complete nor was it permanent. The animals' pupils were di-

[4] Wilson Laboratories.

lated and the eyes were fixed and staring. No interference with the light reflex was apparent.

The effects of a number of compounds, the lack of which have been associated with seizures of hysteria by other workers, were studied with those samples found to produce attacks. The responses of a test animal receiving sample 6 to supplementation with thiamine hydrochloride, casein, dried canned dog food, and lysine hydrochloride are

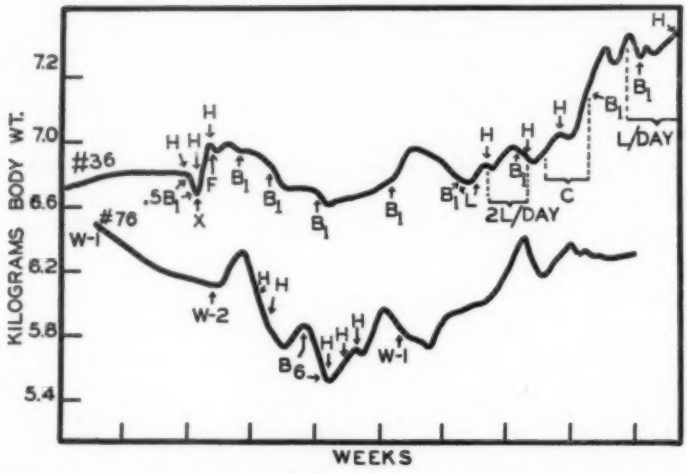

Fig. 1 Growth curves of dogs fed hysteria production rations. H = hysteria attack.

Dog no. 36. Fed dog food sample no. 6 throughout the period shown in the graph. Supplements: $B_1 = 4$ mg. of thiamine hydrochloride; C = 10 gm. of casein per day; X = 200 gm. of canned dried dog food; F = 135 gm. of canned dried dog food; L = 250 mg. of d-lysine dihydrochloride.

Dog no. 76. Fed ration W-1 (casein ration, see table 2) for 2 weeks before receiving wheat gluten ration W-2 for 3 weeks; B_6 represents oral administration of 2 mg. of pyridoxine hydrochloride.

shown in figure 1 (dog no. 36). None of these supplements either prevented or appreciably delayed the onset of attacks. Sample 11 (table 1) still caused hysteria when supplemented with 5% crude casein. The lysine, canned dried dog food, and casein supplements elicted small growth responses. Similar effects were obtained with other dogs.

Canine hysteria from wheat gluten

Several laboratory rations were devised to study the effects of wheat gluten (table 2). Rations W-1 and W-2 are Dr. Parry's modifications of Melnick and Cowgill's rations ('37). The remainder of the rations

have been modified to correspond more closely to the constituents generally used in this laboratory for experimental animals. To reduce the chances of rancidity not more than a week's supply of each ration was mixed at a time. The dogs were given access to 50 gm. of ration per kilogram of body weight per day, which was approximately the average consumption on rations of this type. This restriction was found necessary to prevent the dogs from over-eating when first fed these high sucrose rations. Such gorging is followed by indigestion and a marked drop in food consumption which may persist for several days. Water was supplied ad libitum. Supplements of proteins and amino acids which amounted to more than 1% of the ration were substituted for equal amounts of sucrose.

TABLE 2

Laboratory rations used for wheat gluten studies [1]

CONSTITUENT	W-1	W-2	W-3	W-4	W-7	W-8	W-9	W-10	W-12
Acid-washed casein	4.76	...	..	27.5	20	20	20	20	20
Wheat gluten	...	4.87	30	..	10	15	20	25	..
Lard	3.77	3.77	21	21	21	21	21	21	21
Sucrose	7.50	6.33	40	42.5	40	35	30	25	50
Salts 4	...	...	4	4	4	4	4	4	4
Wesson salts	0.30	0.30	..	..	..	..	..	..	..
Bone ash	0.40	0.40	..	..	..	..	..	..	..
Liver Fraction B (Wilson) [2]	0.33	0.33	3	3	3	3	3	3	3
Cod liver oil	Note [3]	Note [3]	2	2	2	2	2	2	2
Total	17.06	15.90	100	100	100	100	100	100	100

[1] All animals supplemented with 1.5 mg. of thiamine hydrochloride, 2.0 mg. of niacin, 1.0 mg. of pyridoxine hydrochloride, and 10 mg. of alpha-tocopherol per kilogram of body weight per week. Supplements given orally by pipette.

[2] Wilson Laboratories, Chicago, Ill.

[3] Two ml. cod liver oil orally per day by pipette.

Experiment I. Two 6-month-old fox terriers weighing 5.7 kg. and 6.5 kg. were fed ration W-1 (casein) for 13 days. The ration was changed to W-2 (wheat gluten) and the incidence of hysteria attacks observed. The responses of animal no. 76 are shown in figure 1. This dog had an attack after 5 days on the wheat gluten ration. The other suffered from deranged balance by the eighth day and had attacks of hysteria on the tenth day, dying after 22 days on the ration. Supplements of additional crystalline pyridoxine were given orally to both animals without effect.

Experiments II, III, IV. Effects of lysine and protein supplements. II. (fig. 2). Six pups 7 weeks of age were fed ration W-1 (casein) for 9

days. Dogs nos. 78, 80, 81 and 82 were then changed to ration W-2 (wheat gluten). In addition, dogs nos. 81 and 82 received d-lysine dihydrochloride [5] equivalent to 0.6% of the ration. Dogs nos. 79 and 83 were maintained on the casein ration as controls. Control dogs nos. 79 and 83 continued to grow at a good rate and were normal in behavior. The four littermates fed rations containing wheat gluten all had attacks of hysteria 3 days after the change in rations. Six-tenth per cent d-lysine dihydrochloride in the diet did not retard the onset of attacks.

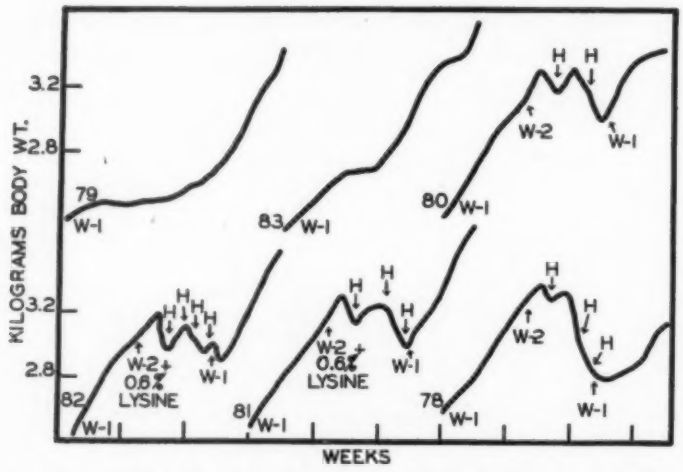

Fig. 2 Supplementation of wheat gluten ration with lysine. Control dogs, no. 79 and no. 83, fed casein ration W-1 throughout experiment. H = hysteria attack.

III. (figs. 3a, 3b). After a 12-day recovery period on ration W-1 dogs nos. 78, 79, 80, 81, 83 and 85 were placed on the following experiment. No. 78 was maintained on the casein ration as a control. No. 81 was fed ration W-2; nos. 79, 80, 81 and 85 received W-2 plus the supplements designated on the graphs. Neither 1.2% d-lysine dihydrochloride, 18% casein, 18% casein hydrolysate, nor 18% gelatin prevented hysteria. The onset of the disease seemed to be slightly delayed in the dogs receiving the protein supplements and the growth rate was increased by the addition of casein or casein hydrolysate. Calcium pantothenate (fig. 3) did not alleviate the symptoms.

IV. After a 9-day preliminary feeding period on ration W-4 (casein), two black Irish-Setter-German Shepherd Cross littermates, nos. 90 and 91, were fed ration W-3 (wheat gluten). The ration of no. 91 was

[5] Merck.

supplemented with 4.97% dl-lysine monohydrochloride. Dog no. 90 developed hysteria in 2 days; dog no. 91 had its first attack in 3 days. The growth of the animal fed lysine was slightly better.

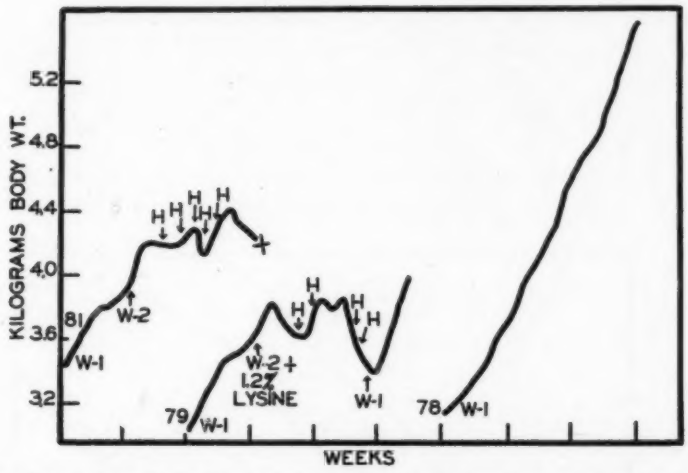

Fig. 3a Effect of supplementing wheat gluten rations. Supplements of 2.5, 2.0 and 10.0 mg. of calcium pantothenate were administered orally to dog no. 79 during the seventh, eighth, and tenth days on ration W-2 plus 1.2% d-lysine dihydrochloride. Dog no. 81 in a similar manner was fed 2.5, 5.0, and 10.0 mg. of calcium pantothenate during the ninth, eleventh, and twelfth days on ration W-2. H = hysteria attack.

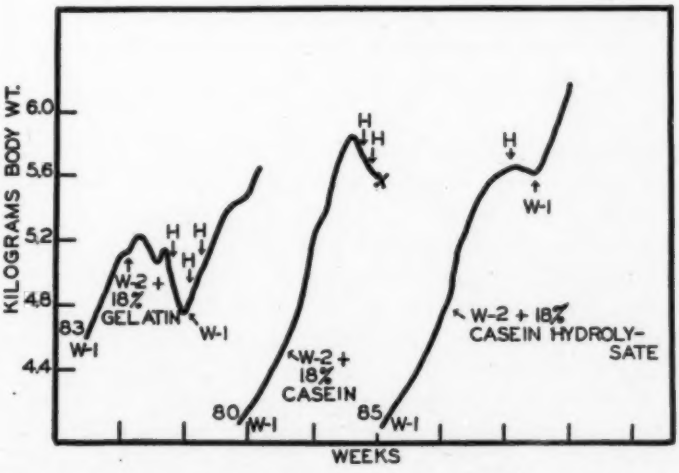

Fig. 3b Effect of supplementing wheat gluten rations. H = hysteria attack.

Experiment V (fig. 4). Effect of various levels of wheat gluten intake. If the effects of wheat gluten were due to a partial deficiency of the protein, one might expect the deficiency to be less acute at higher levels of intake. On the other hand the effects of an imbalance or of a toxicity might be expected to be accentuated by greater consumption.

After a recovery period of 14 days or more, dogs nos. 78, 79, 82, 83, and 84 were fed rations (1) W-12, (2) W-7, (3) W-8, (4) W-9, (5) W-10, respectively, containing 0%, 10%, 15%, 20%, and 25% wheat gluten in addition to 20% casein. The results were as follows: (1) The control animal was maintained on the casein ration in normal condition for 36 days. (2) First hysteria attack was observed after 22 days on the 10%

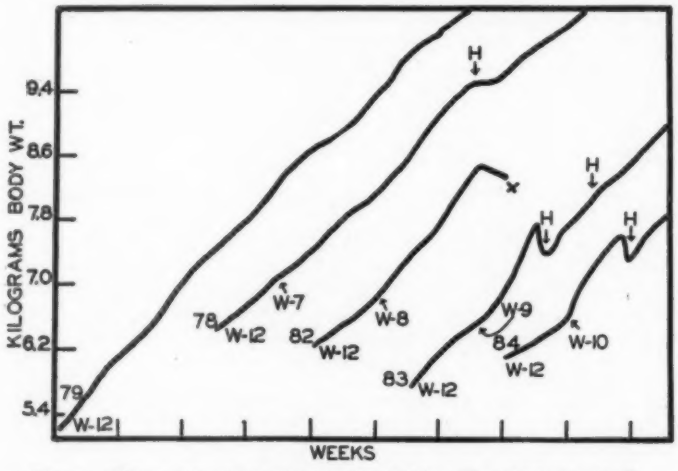

Fig. 4 Effect of level of wheat gluten intake. H = hysteria attack.

wheat gluten ration. (3) Death occurred after 15 days on the 15% wheat gluten ration. (4) First hysteria attack was observed after 9 days on the 20% wheat gluten ration. (5) First hysteria attack was observed after 7 days on the 25% wheat gluten ration.

The hysteria attacks observed in these experiments were similar to those occurring in dogs fed the baked dog foods. Wheat gluten caused hysteria more consistently and, in general, more quickly. Convulsions occurred frequently, varying in severity from brief attacks of a half-minute's duration to a series of convulsions lasting for more than an hour. Post-mortem examination of a number of dogs failed to reveal any morphological changes in the viscera which could be attributed to the feeding of wheat gluten with one exception; in one animal which died

from hysteria the adrenals showed severe hemorrhage in the medullary region. No signs of cerebral hemorrhage or lesions were found.

DISCUSSION

The close correlation between the occurrence of hysteria and the ration fed supports the viewpoint that the disease is dietary in origin. The possibility that any infectious agent is involved seems very remote. Normal animals and animals affected with hysteria were always in close association; usually being separated only by a wire mesh. Attempts to produce toxins on bacterological media inoculated with an organism isolated from wheat gluten or with wheat gluten itself have given negative results and 6 hours autoclaving has failed to reduce the activity of wheat gluten.[6] That the disease arising from the feeding of certain baked dog foods and that derived from the consumption of wheat gluten are a single entity seems probable. The response of test animals seems to be the same in both cases except for the time required for its onset and the difference in severity of attacks. The samples of dog food found to be active contained large amounts of wheat products. Attacks were not prevented in either case by the addition of thiamine, HCl, lysine, casein, casein hydrolysate, or the presence of vitamin A concentrates in the ration.

In the early phases of this work it seemed that we might be dealing with a deficiency. As the work progressed it became increasingly evident that a toxicity was involved. That a protein or an amino acid deficiency did result from some of the baked dog foods is true. Animals receiving such products as the chief constituent of their rations showed a growth response to protein and lysine supplements (fig. 1). Although there is no clear evidence that those supplements reduced the incidence of hysteria attacks, it is conceivable that under such conditions of a combined deficiency and toxicity the correction of the deficiency might increase an animal's resistance to the toxicity. If the toxicity were not too acute, hysteria might even be prevented. This is a possible explanation for the curative effects of casein, lysine (Arnold and Elvehjem, '39), vitamin A (Walston '33) and thiamine (Patton, '39) reported in the past. Although wheat gluten flour is also deficient in lysine, conclusive proof of a toxicity is apparent in the studies with dogs. It has been demonstrated repeatedly that hysteria can be produced by addition of wheat gluten flour to an adequate ration. Furthermore, supplementation of wheat gluten with lysine does not prevent the disease.

[6] Unpublished data.

The disease does not seem related to pyridoxine-deficiency fits of rats and pigs reported by Chick ('40), nor the valine-deficiency staggers of rats observed by Rose ('39).

The nature of the toxic factor is still in question. It appears to be distinct from selenium which has been found to be the toxic substance in certain grains (Moxon, '37). Recent analyses made by Dr. Moxon of the South Dakota Experiment Station show the selenium contents of two samples of our wheat gluten flour to be 1.2 and 2.4 parts per million. Even when these materials were fed as 30% of the rations the intake of selenium was well below the minimum levels reported to be toxic. Balls and Hale ('40) have reported a material extracted from wheat flour by petroleum ether and found to be toxic (Coulson et al., '42) for mice and certain microorganisms. We have not found wheat gluten flour toxic for mice when fed as 30% of the ration.[7]

As a further point of interest it should be mentioned that in preliminary studies we have been unable to detect any of the usual symptoms of canine hysteria in the chick, rat, guinea pig and monkey when fed rations containing large amounts of wheat gluten, for periods of 3 weeks or more.[7] Melnick and Cowgill ('37) have also reported the rat to be immune to the toxic factor in gliadin.

The chemical properties of the toxic material in wheat gluten are now under investigation. Although details of the work cannot be presented here, it is significant that the wheat gluten can be rendered water-soluble by digestion with pancreatin.[7] It does not seem probable that it can be causing any physical interference with gastro-intestinal function in spite of its extremely gummy character previous to digestion.

SUMMARY

Canine hysteria of the nutritional type has been produced in dogs by feeding baked dog foods containing wheat gluten flour and experimental rations containing wheat gluten flour. The disease appears to be caused by some toxic factor present in the wheat products. Although the proteins of these products are deficient in lysine, protein and lysine supplements do not prevent the disease. Hysteria can be caused in dogs receiving nutritionally adequate rations by the addition of wheat gluten flour to the rations.

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EDITORIAL REVIEW

THE ATWATER SYSTEM OF CALCULATING THE CALORIC VALUE OF DIETS

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Some years ago Morey ('36) made a very comprehensive analysis and comparison of methods of calculating the energy value of diets, from which she concluded: "The multiplicity of methods used in calculating the amount of energy actually derived by the body from a given amount of food eaten or purchased makes it extremely difficult to compare accurately different dietary studies and standards." The problems here involved become of special importance at the present time because of the need for a common basis for international comparisons of food supplies and for their evaluation in relation to dietary needs. This review deals with these problems, with particular reference to the applicability of the Atwater system.

In this system, which has long been generally employed in the United States, calories are obtained by multiplying grams of protein, fats and carbohydrates by the factors, 4–9–4, respectively. On the other hand the Rubner factors, 4.1–9.3–4.1, have generally been used in Europe and elsewhere. The principal difference between the two sets of factors is that the Atwater values take account of digestion losses which those of Rubner do not. Atwater has pointed out that Rubner's values for fat and carbohydrate are heats-of-combustion values and that when they are multiplied by the appropriate coefficients of digestibility they become approximately the same as his own (Atwater's). Applied to mixed diets Rubner's factors give a caloric value which is about 3% higher than obtained by Atwater's factors, according to Morey ('36) who has reviewed the bases of the two sets of values.

There is a further difference in the practice in the U.S.A. and in the U.K. (United Kingdom). According to the Atwater system the carbohydrate factor 4 is applied to carbohydrates as determined "by difference", whereas the British workers, McCance and Widdowson ('42)

make the calculation on the basis of what they designate as the "available carbohydrate", namely, the directly determined starch, sugars and dextrins. Further they express all of these carbohydrates as glucose and multiply the total by 3.75, the heat-of-combustion value of this sugar, to obtain calories.

More recently the Accessory Food Factors Committee of the Medical Research Council of the U.K. have used the Atwater factors in computing the composition of war-time foods but they have expressed the "available carbohydrates" in terms of their starch equivalents (disaccharides $\div 1.055$, hexoses $\div 1.11$) before applying the factor 4, whereas by the Atwater system the total carbohydrates are multiplied by the factor directly. Thus the M.R.C. (Medical Research Council) procedure results in lower values for all foods containing sugars. Cane sugar, for example, has approximately 5% fewer calories by the M.R.C. calculation than by the Atwater method.

The problems arising from the discrepancies between the U.K. procedure and the Atwater system as used in the U.S.A. and Canada became apparent in a joint study made by experts of the three countries, involving a comparison of food consumption levels in relation to nutritional needs. The difficulties encountered are discussed in Appendix D of the Report of the Special Joint Committee ('44). The problem has arisen again in studies in which U.K. and U.S.A. scientists are cooperating in evaluating the food supplies of various countries of continental Europe, as well as in connection with the activities of the United Nations Relief and Rehabilitation Administration (U.N.R.R.A.). It will continue to be a problem, post-war, for international programs in the field of food and nutrition unless a common basis for food evaluation is agreed upon. The present article is presented as a contribution to this objective. The experimental basis of the Atwater system is reviewed, with particular reference to the points of difference with the present U.K. procedure. Its applicability to diets differing widely in make-up from the U.S. diet is critically discussed.

Atwater ('00) has summarized the experimental basis of his procedure in the 12th Annual Report of the Storrs Agricultural Experiment Station. The detailed supporting data are published in other reports from this station, in bulletins of the Office of Experiment Stations and elsewhere. For purposes of the present study and because the publication in question is doubtless unavailable in many libraries, table 10 from the Storrs Report is here reproduced without change (table 1).

Atwater first summarized 185 dietary studies made on different groups of people in different areas of the U.S.A. He classified the foods

TABLE 1

Factors for heats of combustion and fuel values of nutrients in different groups of food materials and in mixed diet.

(Table 10 from Atwater, Report of Storrs Agricultural Experiment Station for 1899.)

KIND OF FOOD MATERIAL	NUTRIENTS FURNISHED BY EACH GROUP PER 100 GRAMS TOTAL	HEAT OF COMBUSTION PER GRAM	PROPORTION OF TOTAL NUTRIENT ACTUALLY AVAILABLE	TOTAL ENERGY PER GRAM IN AVAILABLE NUTRIENTS	FUEL VALUE	
					Per gram available nutrients	Per gram total nutrients
	A	B	C	D =(B × C)	E [1]	F [2]
Protein	*Gm.*	*Cals.*		*Cals.*	*Cals.*	*Cals.*
Meats, fish, etc.	43.0	5.65	.97	5.50	4.40	4.25
Eggs	6.0	5.75	.97	5.60	4.50	4.35
Dairy products	12.0	5.65	.97	5.50	4.40	4.25
Animal food	61.0	5.65	.97	5.50	4.40	4.25
Cereals	31.0	5.80	.85	4.95	4.55	3.70
Legumes	2.0	5.70	.78	4.45	4.45	3.20
Vegetables	5.5	5.00	.83	4.15	3.75	2.90
Fruits	0.5	5.20	.85	4.40	3.95	3.15
Vegetable food	39.0	5.65	.85	4.80	4.40	3.55
Total food	100.0	5.65	.92	5.20	4.40	4.00
Fat						
Meat and eggs	60.0	9.50	.95	9.00	9.50	9.00
Dairy products	32.0	9.25	.95	8.80	9.25	8.80
Animal food	92.0	9.40	.95	8.95	9.40	8.95
Vegetable food	8.0	9.30	.90	8.35	9.30	8.35
Total food	100.0	9.40	.95	8.90	9.40	8.90
Carbohydrates						
Animal food	5.0	3.90	.98	3.80	3.90	3.80
Cereals	55.0	4.20	.98	4.10	4.20	4.10
Legumes	1.0	4.20	.97	4.05	4.20	4.05
Vegetables	13.0	4.20	.95	4.00	4.20	4.00
Fruits	5.0	4.00	.90	3.60	4.00	3.60
Sugars	21.0	3.95	.98	3.85	3.95	3.85
Vegetable food	95.0	4.15	.97	4.00	4.15	4.00
Total food	100.0	4.15	.97	4.00	4.15	4.00

[1] Values for fats and carbohydrates, same as corresponding values in column B. Values for protein, same as corresponding values in column B minus 1.25.

[2] Values for fats and carbohydrates, same as corresponding values in column D. Values for protein, same as corresponding values in column D minus 1.25.

consumed into various groups (meats, milk, cereals, sugar, vegetables, etc.), and calculated the percentage of protein, fat and carbohydrate furnished by each group, thus providing average figures for the 185 diets. These average figures were taken to represent the relative proportions of total food and total nutrients supplied by the different food groups in the "average diet". The amounts of the nutrients calculated to be furnished by the various foods of this diet are given in column A of the table.

Atwater next tabulated the heats-of-combustion (gross caloric values) of various proteins, fats and carbohydrates, and on the basis of these data he assigned values for the different groups, as shown in column B of the table. Based on the distribution of these groups in the average mixed diet, obtained from the dietary studies, he calculated that the heat-of-combustion value for the protein of such a diet could be considered as 5.65 cal. per gram, for the fat 9.4 and for the carbohydrates 4.1. Atwater summarized the data of ninety-seven human digestion experiments with mixed diets and of others with single food stuffs, to arrive at the average coefficients of availability (digestibility) for the nutrients of the different food groups and for the average mixed diet, as is shown in column C. Thus for this diet the figures became: protein 92; fats 95; carbohydrates 97. In view of the different meanings given to the term "availability" by others, it should be noted that Atwater used the term to denote digestibility.

To arrive at the calories which were considered useful for growth, maintenance and repair, Atwater applied the coefficients of availability (column C) to the heats-of-combustion (column B) for the various foods, thus obtaining the data in column D. Then he corrected these data for 1.25 cal. per gram of protein lost as urea and thus arrived at the final figures in column F. The data in column E do not enter into this calculation. These are the heats-of-combustion values, less 125 cal. per gram in the case of protein. They are the values which should be applied to digested nutrients. This would result in the same figures as applying the values in column F to the grams of total food (column A). The latter calculation is the one we actually employ in evaluating diets and the term "fuel value" is now used specifically to denote the values in column F. Here again it should be noted the values thus assigned to a given nutrient differ widely for different food groups. For example, the protein of meat has a value of 4.25, while for cereals the value is 3.7. Based upon the proportion of the different food groups found in the average diet he arrived at the overall values we now use, namely: protein 4, fat 9 (rounded off from 8.9); and carbohydrates 4.

This analysis of the basis of the Atwater system, and a review of the supporting experiments, indicate that it rests upon a large body of scientific data, both chemical and physiological, carefully and accurately determined. The analysis also reveals certain facts regarding the system which need to be kept in mind in its application.

It should be noted that the Atwater factors, 4–9–4, as arrived at by the procedure indicated in the table, apply strictly only to the diets in which the kinds and proportions of foods are similar to those of his average mixed diet. A comparison of the make-up of the latter with the present day U.S.A. average diet, as revealed by the Consumption Levels Study ('44), indicates that the factors are currently applicable in the U.S.A. They tend to overvalue, however, diets which contain a larger proportion of their calories from the less digestible foods, as is discussed later.

The Atwater values arrive at the calories which are actually utilizable in metabolism, assuming that a digestion trial which measures "disappearance" in the tract is a reliable measure of the absorbed nutrients and assuming that these nutrients are all energy formers (subject to the deduction of the energy of urinary nitrogen). There appears to be no modern physiological evidence to invalidate this assumption in any significant way. The question as to whether the Atwater values rest on reliable digestion data also enters. An examination of the basic data and a comparison of them with the results of more recent studies indicate that the basis for the Atwater values may generally be considered as sound. The values do not measure the "net energy" as conceived by Kellner and Armsby, for they do not take account of heat losses which vary for the different nutrients, as influenced by various factors.

It is apparent from the discussion of the data in the table that the Atwater system takes account of the fact that a certain fraction of the total carbohydrates and of the protein and fat as well, of each food is unavailable, by appropriate deductions based on actual digestion studies, before arriving at the overall factor of 4 to be applied to the total. Thus the M.R.C. procedure of applying the factor 4 to "available carbohydrate" involves a double deduction. The Atwater gross factor of 4.15 (average for all foods) is the one that should be applied here, according to the Atwater system. The M.R.C. system of converting sugars to starch before applying the factor makes the deduction a triple one in the case of sugars. Atwater's overall factor of 4 is not a starch factor. It represents a weighted average for the various carbohydrates as they occur in the diet, thus taking account of the fact that sugars have a lower caloric value than starch. If all are converted to

starch the correct factor by Atwater is 4.1 on the "total" basis or 4.2 on the "available" basis. Thus it appears that the M.R.C. procedure of applying the Atwater factor only to "available carbohydrate" and of calculating sugars back to starch before applying the value is not in accord with the basis on which the value rests.

While it is agreed that it is chemically more accurate to determine carbohydrates directly, as is the case for "available", than by difference, as is the case for "total", one needs assurance that what is chemically determined as "available" truly represents the physiologically utilizable fraction. Atwater's determinations have this merit at least insofar as digestibility may be considered a proper measure. It is recognized that the "available" values of the M.R.C. do not take account of all the glycogen forming material included with the "total", such as certain organic acids in fruits and vegetables and lactic acid in cheese. Further, with certain foods, physiological evidence indicates that there are significantly more digestible carbohydrate calories present than are measured chemically as starch and sugar. These differences may seem inconsequential but they should be considered before decision is reached that the "available" basis is definitely better. In current U.S.A. tables, as explained in connection with those of Chatfield and Adams ('40), the total carbohydrate values for certain foods, for example, soybeans, have been scaled down where evidence has shown them to contain so much unavailable material that Atwater's coefficient of digestibility tends to overrate them.

The potato, which makes a large contribution to the caloric intake in nearly all countries, is an example of foods about which there is doubt as to whether the starch and sugar are the sole source of utilizable carbohydrate calories. Analytical data for potatoes from various sources indicate that the sum of the starch and sugar present is only about 85% of the total carbohydrates as determined by "difference". Thus the caloric value of potatoes as calculated by the Atwater system is discounted by over 10% when only the "available carbohydrate" is considered. Yet extensive human data reviewed by Rubner and Thomas ('18) indicate that less than 6% of the gross calories of potatoes are lost in digestion, which is in line with losses allowed for in the Atwater system. It appears, therefore, that potatoes must contain utilizable carbohydrate calories in addition to those calculated from "available carbohydrate" and that the latter basis is incorrect for this food. There are even larger differences between the total and the "available" carbohydrates in the case of various pulses and nuts and of most of the com-

mon vegetables. That the carbohydrate represented by these differences is entirely unavailable has not been proved.

It has been mentioned that the Atwater factors for a mixed diet do not apply accurately to many individual foods. While the factor 4 applies to the mixture of proteins in the U.S. diet, the value for eggs is 4.35, meat 4.25, cereals 3.7, legumes 3.2, etc. Similarly the carbohydrate factors for individual foods range from 4.1 for cereals to 3.6 for fruits. Thus, the fuel values for certain individual foods as they appear in tables of food composition, calculated by the use of the average factors, 4–9–4, can be considered only approximations. The errors here tend to balance out in calculating the fuel values of mixed diets similar to the average diet upon which the factors are based and thus are of no great concern. But this is not true for certain food combinations and the errors involved in the individual values should be recognized in connection with controlled experiments with diets which vary markedly in make-up from the pattern on which the overall values, 4–9–4, are based. For example, if one desired to compare a diet of animal products with a diet of cereals, legumes, and vegetables, at the same caloric level, and used the factors, 4–9–4, for both combinations, the desired caloric equality would not be achieved.

The real problem in the use of the Atwater factors arises in connection with diets which are markedly different in food make-up from the average mixed diet upon which they are based. Compared with the latter, the U.K. wartime diet is lower in animal products and higher in potatoes and cereals. Thus the Atwater factors would tend to overvalue it because animal protein has a higher fuel value than vegetable protein. The cereal carbohydrate of the Atwater diet came very largely from highly milled flour, as is the case for the present U.S.A. diet. The flour of the U.K. wartime diet, however, is an 85% extraction, and its energy is somewhat less digestible than the highly milled products.

Thus, on this basis, the Atwater factors tend to overvalue the calories of a diet such as the present U.K. combination which contains over one-third of its calories from this somewhat less digestible source. These considerations undoubtedly influenced U.K. authorities in adopting their present procedure. The overvaluation which might result from the use of the factors, 4–9–4, becomes of greater concern in connection with the wartime diets of occupied countries which consist much more largely of foods of plant origin, and particularly of less refined foods higher in fiber and thus less digestible. For the same reasons their application to the usual diets of Eastern peoples is subject to error.

Atwater never intended that the factors, 4–9–4, should be applied without modification to all diets. His data reproduced in the table provide the basis for deriving other sets of overall factors for any combination of the food groups and thus for any diet made up largely of the groups listed. As an alternative, the individual fuel value for each nutrient of each food or food group, as listed in column F, could be applied to the corresponding nutrient supply data to arrive at caloric values by foods or food groups, and their values could then be totalled to give the calories for the diet as a whole. In this way the Atwater system could be applied to any diet, without introducing the errors inherent in the average factors for diets differing widely from the original average mixed diet. For either of these procedures a figure for vegetable fats as such, not provided by the Atwater table, is required. It would seem that they should be assigned the fuel value of 9, the same as animal fats, rather than the figure of 8.35 which Atwater lists for fats in "vegetable food". Further, factors would need to be provided for cereals of low extraction. There are digestion data in the literature which could furnish the basis for such factors.

The above procedures would of course greatly increase the calculations required compared with the present use of a single set of factors for the diet as a whole. A short-cut procedure which would correct for significant deviations in the proportions of foods of plant and animal origin from the pattern of the average mixed diet would involve a reweighting of the average values accordingly. Atwater's overall fuel value of 4 for protein is based upon an approximately 60:40 distribution of animal protein averaging 4.25 cal. per gram and of vegetable protein averaging 3.55 cal. A 30:70 distribution would give an overall value of 3.75. Thus, for any diet an appropriate overall value for protein could be calculated by weighting Atwater's values for the two classes of protein in accordance with the proportions of each present. Similarly, appropriate values could be computed for fat and carbohydrates. This procedure would not take account of variations caused by large and significant differences in food distribution within the two groups.

Thus, another alternative might involve a condensation of the data of the groups of the original Atwater table to provide values such as those listed in table 2. Here values for cereals of 85% extraction have been included, based upon digestion studies of the U.K. wartime flour. In applying the data in this table to the food supply for a given country, the overall value for calculating its protein calories could be easily derived as an average of the individual values, weighted in accordance

with the proportions of the different classes of foods present. Similarly, an appropriate value for fat could be obtained. It would be little different from the present Atwater value because such a small portion of fat is supplied from foods other than animal products and animal and vegetable fats as such. Similarly, an appropriate value to be applied to "total" carbohydrates would not be significantly lower than 4, except for diets, for which a substantial proportion of the carbohydrate calories is furnished by cereals of low extraction. The table could be condensed, or made more detailed. A study of Atwater's basic data might modify some of the values. They serve here merely to illustrate one possible solution of the problem.

TABLE 2

Condensation of original Atwater table of fuel values of nutrients in different groups of food materials.

	PROTEIN	FAT	TOTAL CARBOHYDRATES
Animal products	4.25	9.00	3.8
Animal and vegetable fats		9.00	
Cereals (70% extr.)	3.70 —		4.1
(85% extr.)	3.60		3.8
Pulses and nuts	3.20 —	8.35	4.0
Vegetables (including potatoes)	2.90		4.0
Fruits	3.15 —		3.6
Sugar (sucrose)			3.85

Any of these procedures could be modified to utilize the "available carbohydrate" basis, and the gross caloric values in column E of Atwater's table, if that were deemed desirable. For reasons discussed in this review the writer does not favor the adoption of this basis at the present. Particularly, certain procedures employed in its use by the M.R.C. seem questionable if the experimental basis of the Atwater system is accepted.

The possible procedures here outlined for adapting the Atwater system to diets differing widely from those upon which the factors, 4–9–4, were derived increase the computations which must be made. How far it is worthwhile to attempt to refine the original procedure, in view of errors inherent in estimating food supplies and of the variability represented in data as to "average composition", may be questioned. But for international comparisons, in which there are wide differences in the character of the food supplies, some modifications from the use of a single set of overall factors, but still having a common basis, seem essential. Unless there is a comparable basis for computing calorie

supplies the use of any common standard as to calorie allowances or requirements, as a measure of the adequacy of these supplies, is meaningless. There can be no worthwhile international studies without some common agreement as to procedures. It is hoped that the information provided by the present review of the experimental basis of the Atwater system may prove helpful in achieving this end insofar as food and nutrition programs are concerned.

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MEAD JOHNSON AND COMPANY 'B-COMPLEX' AWARD

Nominations are solicited for the 1945 Award of $1,000 established by Mead Johnson and Company to promote researches dealing with the B complex vitamins. The recipient of this Award will be chosen by a Committee of Judges of the American Institute of Nutrition and the formal presentation will be made at the annual meeting of the Institute at Cleveland on May 8, 1945.

The Award will be given to the laboratory (non-clinical) or clinical research worker in the United States or Canada who, in the opinion of the judges, has published during the previous calendar year January 1st to December 31st the most meritorious scientific report dealing with the field of the 'B-complex' vitamins. While the award will be given primarily for publication of specific papers, the judges are given considerable latitude in the exercise of their function. If in their judgment circumstances and justice so dictate, it may be recommended that the prize be divided between two or more persons. It may also be recommended that the award be made to a worker for valuable contributions over an extended period but not necessarily representative of a given year. Membership in the American Institute of Nutrition is not a requisite of eligibility for the award.

To be considered by the Committee of Judges, nominations for this award for work published in 1944 must be in the hands of the Secretary by January 15th, 1945. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate the task of the Committee of Judges in its consideration of the nomination.

ARTHUR H. SMITH
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SECRETARY, AMERICAN INSTITUTE OF NUTRITION

BORDEN AWARD IN NUTRITION

The American Institute of Nutrition will make this award in recognition of distinctive research by investigators in the United States and Canada which has emphasized the nutritive significance of the components of milk or of dairy products. The award will be made primarily for the publication of specific papers, but the judges may recommend that it be given for important contributions over an extended period of time. The award may be divided between two or more investigators. Employees of the Borden Company are not eligible for this honor.

The formal presentation will be made at the annual meeting of the Institute at Cleveland, May 8, 1945. To be considered for the award, nominations must be in the hands of the Chairman of the Nominating Committee by January 15, 1945. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate consideration for the award.

FREDERICK J. STARE
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Boston, Massachusetts

CHAIRMAN, NOMINATING COMMITTEE

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